High Root Temperatures:
A Buried Threat to Plant Growth

A THESIS
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE

Dr. John E. Erwin, Advisor

May 2019

Acknowledgements

First and foremost I would like to acknowledge the significant contribution of my advisor, Dr. John Erwin, to my development as a master of science in horticulture. Additionally, I would like to thank my other advisory committee members, Drs. Michael Sadowsky, Julie Grossman, and Eric Watkins, for their important insight and guidance over the last two years.

The managers and staff of the UMN Plant Growth Facility – Pam Warnke, Doug Brinkman, Roger Messner, Dean Ziertman, and Tha Cha – also have my deepest gratitude for their enduring patience and flexibility throughout the course of my numerous pop-up projects and environmental micro-managing.

I would also like to thank Dr. Bruce Bugbee & Paul Kusuma of Utah State University, Ron & Nick Wagner of Wagner's Greenhouse, Jerry & Jason Fischer of Orchids Limited, and Drs. Rusty Rodriguez and Regina Redman of Symbiogenics for their generous contributions of time, support, and materials to my research projects.

My Alderman 420 officemates deserve recognition for both their wisdom and perpetual supply of freshly-brewed coffee, as well as all of my other Applied Plant Sciences peers who have made this a unique and thought-provoking journey.

And last, but certainly not least, I would like to acknowledge the friends and family who have supported me through all of the challenges I've faced over the last two years. I couldn't have kept all of the moving pieces together without their limitless support along the way. Especially Sarah, who saw me through even the toughest times with patience and understanding.

Dedication

This thesis is dedicated to my parents, for always encouraging my curiosity in the natural world and humoring my hobbies every step of the way.

And to Grandma Bets, whose kitchen table orchid collection first inspired my fascination with plants and ultimately my career in horticulture.

Abstract

Growing plants in containerized systems can result in high root temperatures (HRT) as containers, media, and roots above the ground are exposed to air and sunlight, commonly experiencing temperatures over 50°C. Damage caused by HRT and associated consequences for growth are not well characterized amongst herbaceous plants. The research in this thesis evaluated how HRT impacted physiological and morphological responses of eight tomato (*Solanum lycopersicum*) varieties characterized as 'heat-tolerant' or 'sensitive' based upon aboveground traits.

The first pair of experiments quantified respiration rates and electrolyte leakage of excised whole root masses in response to acute HRT exposure between 48 and 62°C. Root respiration rates increased from 21.6 μmol hr^{-1} g^{-1} at 48°C to 26.9 μmol hr^{-1} g^{-1} at 51°C, and then decreased to approximately 0 μmol hr^{-1} g^{-1} at 57°C. Varieties did not differ in responses to root temperature. Root temperature and variety interacted to impact proportional electrolyte leakage, which increased across varieties between 50 and 54°C. Results of these experiments suggested that critical physical and metabolic damage occurs to tomato roots at ≥50°C.

For the second pair of experiments, morphological and photosynthetic responses of two tomato varieties previously characterized as heat-tolerant ('Solar Fire') or -sensitive ('Amana Orange') were assessed. Plants were grown at root temperatures ranging from 25 to 60°C for 8 h^{-1} d^{-1} over 10 d, and differences in morphology were noted. Plant height and leaf size decreased as temperature increased. Shoot and root fresh and dry mass gain decreased when RT increased from 35 to 50°C. 'Solar Fire' and 'Amana Orange' did not differ in fresh and dry mass gain responses or percent

reduction in shoot and root mass gain. Root masses of 'Solar Fire' and 'Amana Orange' were also heated to 55°C for 260 min in the afternoon of one day and plants were evaluated for changes in leaf photosynthetic rate and stomatal conductance the following four days. Photosynthetic rate and stomatal conductance decreased after one 55°C RT exposure for 4 d compared to plants maintained at 25°C. 'Solar Fire' and 'Amana Orange' differed in percent reduction in stomatal conductance. The results suggested diurnal, short-term HRT negatively impacted growth and photosynthesis regardless of reported above-ground heat tolerance, and that even one supraoptimal HRT event could reduce photosynthetic activity for days.

Lastly, five root-associated fungi and bacteria (*Azospirillum brasiliense, Bacillus amyloliquifaciens, Curvularia protuberata, Glomus intraradices,* and *Trichoderma harzianum*), thought to confer increased resistance to biotic and abiotic stresses, were explored for their potential to alleviate HRT effects on tomato growth. 'Amana Orange' seedlings were inoculated with the before-mentioned microbes and exposed to root temperatures between 35 (control) and 55°C (HRT) for 8 h^{-1} d^{-1} over a 10 d period. Plant height and shoot, root, and total plant fresh and dry mass decreased as root temperature increased from 35 to 50°C. Dry mass gain of roots and shoots did not differ between un-inoculated and inoculated plants, but some differences were observed between inoculant species. The results suggested HRT have detrimental effects on above- and below-ground tomato growth and inoculation with the before-mentioned organisms did not alleviate those negative effects.

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Chapter 1

High Root Temperatures:

An Invisible Stressor Reviewed

Damage caused by high root temperature and associated consequences for herbaceous plant growth are not well characterized. High root temperature (HRT) increases root respiration and reactive oxygen species concentration, reduces cellular integrity and contributes to abscisic acid biosynthesis, impacting leaf photosynthesis. Mechanisms contributing to root HRT tolerance such as carbohydrate allocation and antioxidant production are subjects of focus with emphasis on trait-selective breeding and root stress alleviation by application of biological inoculants to media. This review highlights plant responses to HRT, factors associated with root thermotolerance, and the potential alleviation of HRT stress by application of root-associated microbes.

Table 1.1. General Concepts

- High root temperatures can reduce plant growth.
- Increased root respiration can decrease carbohydrate status and increase reactive oxygen species synthesis.
- Plant thermotolerance is related to carbon allocation to proteins and lipids and increased antioxidant activity after reactive oxygen species accumulation.
- Bacterial and arbuscular mycorrhizal fungal inoculants can improve growth of plants exposed to high root temperatures.

Table 1.2. Glossary of Terms and Abbreviations

- **Abscisic acid (ABA)** – Key hormone associated with developmental and stress signaling activities in plants.
- **Arbuscular mycorrhizal fungus (AMF)** – Any of a wide variety of fungal endophyte taxa that form symbiotic relationships with plant root systems and perform a variety of functions for roots including nutrient and water acquisition.
- **Alternative oxidase pathway (AOX)** – The respiration pathway utilized by mitochondria to generate ATP from available carbohydrates, often under stress conditions.
- **Cytochrome C oxidase pathway (COX):** The respiration pathway commonly used by mitochondria to process carbohydrates into ATP.
- **Stomatal conductance (G_s)**: A measure of stomatal gas exchange.
- **Hydrogen peroxide (H_2O_2):** The less toxic reactive oxygen species formed after the reduction of superoxide by antioxidants.
- **Malondialdehyde (MDA):** A product of lipid damage by peroxidation by reactive oxygen species.
- **Superoxide (O_2-):** A toxic reactive oxygen species produced during the respiratory process of mitochondria.
- **Photosynthetic rate (P_n)**: A measure of the rate plants convert CO_2 and light energy into carbohydrates.
- **Q_{10}:** The rate respiration increases for every 10°C change in temperature.
- **Reactive oxygen species (ROS)** – Chemically reactive compounds that, in small concentrations, are associated with cellular signaling, but which in large concentrations can damage lipids and proteins.
- **High root temperature (HRT)**: Root temperatures exceeding the optima for growth and cellular activity.

Introduction

Vegetable and ornamental crops are often grown in containers in commercial production in areas where plants can't be grown outdoors year-round. Such containerized plants are commonly grown above-ground in outdoor fields or in greenhouses where solar radiation can heat pots, media and roots to above air temperatures during the day. When solar radiation is high and day lengths are long, media and root temperatures (hereafter RT) can heat to levels that negatively impact plant growth. Hydroponic and aeroponic systems in greenhouses can be similarly heated, with elevated temperatures in these systems associated with altered nutrient uptake, plant growth reduction and increased disease proliferation and/or susceptibility (Dodd *et al.* 2000; He *et al.* 2001; Benlloch-González *et al.* 2017; Falah *et al.* 2010). Raised bed systems commonly used to grow vegetables and some fruit, particularly when covered with dark plastic mulch or polyethylene, can also have media and RT higher than soil temperatures (Locher *et al.* 2003; Teasdale & Abdul-Baki 2019). Taken together, stress associated with high root temperatures may negatively impact plant growth and yield more than appreciated by scientists and agricultural crop producers.

The term 'heat stress' has been defined by Wahid et al. (2007) as the exposure of plant tissue to temperatures 10-15°C above optimal temperature. However, even small temperature increases above growing optima temperatures can have significant consequences for plant metabolic activity and growth. For example, exposure of lettuce (*Lactuca*) varieties to air temperatures ranging from 28 to 36°C for 25 days reduced root and shoot fresh weight compared to plants grown at 25°C (Lai & He 2016). Changes in stomatal density and distribution and reduced in photosynthetic rate (P_n) amongst

blueberry cultivars occurred at air temperatures 5-10°C above day/night temperature optima (Hao *et al.* 2019). Therefore, what can be described as 'heat stress' can occur in a narrower temperature window than what Wahid et al., (2007) suggested.

Plant heat tolerance is often characterized by assessing the impact of high temperature on fresh and/or dry weight, P_n and/or yield (Li *et al.* 2016; Nankishore & Farrell 2016; Xu *et al.* 2017; Zhou *et al.* 2017; Poudyal *et al.* 2018; Hao *et al.* 2019). However, roots can exhibit higher sensitivity to high temperatures than aboveground tissues (Tahir *et al.* 2008; Sailaja *et al.* 2014). While high air temperature stress-effects on stem and leaf growth and photosynthesis (P_n) are well-studied, the impacts of high root zone temperatures on both root physiology and above-media growth are not as well known.

Early studies on high temperature induced root stress focused primarily on the short-term responses of woody plant species (Ingram *et al.* 1986; Foster *et al.* 1991; Martin *et al.* 1991; Sibley *et al.* 1999). Subsequent studies on root and whole plant responses to high RT have expanded to herbaceous plant species and explored the effects of extended, sub-lethal temperature exposures on roots rather than short-term high temperature exposures (Xu & Huang 2000; Huang *et al.* 2012; Rachmilevitch *et al.* 2015; Aidoo *et al.* 2016). Yet the short-term high temperature thresholds and associated physiological responses of the majority of container-grown herbaceous crops remain unknown.

High RT can directly damage roots, while sub-lethal RT may contribute to long-term negative impacts on root growth, and/or whole plant metabolism and growth. Temperatures that exceed the optimal temperature range for roots can cause direct

damage in a short period of time (Ingram *et al.* 1986; Lyles *et al.* 1992). For example, exposure of hibiscus (*Hibiscus rosa-sinensis* 'Kona') to RT of 50°C for 20 min resulted in discoloration of 80% of exposed roots (Lyles *et al.* 1992). Root respiratory activity and reactive oxygen species (ROS) synthesis (concentration) play a role in root temperature sensitivity (Rachmilevitch, Lambers, *et al.* 2006). Additionally, high RT can reduce carbon exchange rates and growth when temperature is increased above optimal temperatures (Du & Tachibana 1994a; Xu & Huang 2000; Nada *et al.* 2003; Monje *et al.* 2007). Respiratory acclimation, the use of alternative respiration pathways and ROS management, therefore, are important components of root tolerance of high temperatures. Characteristics of thermotolerance in species indigenous to high soil temperatures environments has provided some insight into the contribution of these physiological mechanisms for root thermotolerance (Huang *et al.* 2012; Rachmilevitch *et al.* 2015; Xu *et al.* 2015). For example, effective carbon partitioning under high RT stress improves the thermotolerance of the bentgrass *Agrostis scabra* over its relative, *A. stolonifera* (Rachmilevitch *et al.* 2015).

For plant species lacking root thermotolerance, physical reduction of solar radiation is needed to alleviate high temperature stress. Light colored containers and alternative materials to plastic can contribute to reductions in RT (Markham *et al.* 2011; Nambuthiri *et al.* 2015), but cost and labor-effective means of countering temperature stress remain underemployed. One approach, the application of beneficial root-associated microbes, holds promise. Recent research shows that root-associated microbes can ameliorate a variety of abiotic stressors including drought, saline soils, and suboptimal temperatures (Luis M. Márquez 2007; Chowdhury *et al.* 2013; Abd El-Daim *et al.* 2014;

Mona *et al.* 2017). However, the potential role of root-associated beneficial microbes in high RT responses remains largely unexplored. Ancillary research suggests microbial inoculants such as arbuscular mycorrhizal fungi (AMF) might contribute to plant thermotolerance through mechanisms such as antioxidant production and stress hormone suppression (Zhu *et al.* 2010; Matsubara *et al.* 2014; Duc *et al.* 2018).

This review presents 1) our current understanding of root and whole-plant responses to high RT, 2) mechanisms that may be underlying differences in thermotolerance between species and cultivars, and 3) how plant-bacteria/fungal interactions may reduce negative impacts of HRT on crop growth in containerized systems.

Plant Responses to HRT

Increased root respiration at HRT reduces both root and aboveground growth and alters within-plant carbon cycling. Root respiration rates double for every 10°C increase in RT (known as the Q_{10}) to maximum threshold temperature (Atkin & Tjoelker 2003). Bentgrass (*Agrostis stolonifera* and *A. scabra*) root respiration rates increased and root fresh and dry weight decreased at RT greater than 30°C (Huang & Xu 2000; Rachmilevitch, Lambers, *et al.* 2006; Lyons *et al.* 2007). Creeping bentgrass (*A. scabra* and *A. stolonifera* 'Penncross') ^{14}C partitioning to shoots decreased when RT increased from 20 to 37°C (Rachmilevitch *et al.* 2015). ^{14}C translocation to tomato roots (*Solanum lycopersicum v.* 'Vendor') increased when temperature increased from 20 to 35°C, but accumulation in root tissues was low at 30°C because of elevated respiration (Hurewitz & Janes 1983). Similar changes in ^{14}C translocation and respiratory loss were observed for

cucumber plants (*Cucumis sativus*) grown for 6 d at 38°C (Du & Tachibana 1994b). Spring wheat *(Triticum spelta* v. 'USU-Apogee') grown with 28-35°C RT were shorter and had reduced leaf size and increased carbon partitioning to stems and seed heads (Monje *et al.* 2007). Relative growth rate of hydroponically-grown olive (*Olea europaea* 'Arbequina') roots grown at 37°C was reduced 40% after 7 d and after 33 d, leaf dry weight was 39% lower than plants grown at 25°C RT (Benlloch-González *et al.* 2017). Cucumber (*Cucumis sativus)* grown for eight days or more with a 38°C RT had altered root morphology and reduced leaf, stem and root dry weight compared to plants grown at 25-35°C (Du & Tachibana 1994a).

Respiration at high temperatures can be limited by substrate and adenylate availability for energy conversion (Atkin & Tjoelker 2003). When respiratory carbon demand exceeds supply from photosynthesis, plant mass decreases. For instance, exposure to 35°C RT resulted in carbon consumption by respiration exceeding carbon production by photosynthesis in creeping bentgrass (*A. stolonifera* 'L-93' and 'Penncross'), accompanying dry weights that were lower than that of plants at 25°C RT (Xu & Huang 2000). Shoot dry mass of tomato plants (*Solanum lycopersicum* 'Jet Star') grown with a 36°C RT for 19 d was less than that of plants grown at 25°C RT (Klock *et al.* 1997). In that same work, root respiration at 36°C initially increased, but decreased to less than that of plants grown with a 25°C RT suggesting an exhaustion of available carbohydrates. Non-structural carbon content was greatly reduced in rice (*Oryza sativa*), creeping bentgrass (*A. stolonifera* and *A. scabra*), and cucumbers (*C. sativus*) grown for prolonged periods of time at elevated RT (Du & Tachibana 1994a; Xu & Huang 2000; Lyons *et al.* 2007; Arai-Sanoh *et al.* 2010a).

High RT can decrease leaf photosynthetic rate (P_n) and exacerbate carbon depletion in plants when combined with aforementioned increased root respiration rates. Mechanisms underlying high RT impacts on leaf P_n are unclear: cell membrane damage contributing to decreased stem cell water potential, increased root abscisic acid (ABA) synthesis and translocation to leaves where it reduces stomatal conductance (g_s), and reduced photosystem efficiency (F_v/F_m) may all contribute to high RT-limited photosynthesis.

There is an inverse relationship between RT and exposure time on the degree of direct damage to root tissues (Ingram *et al.* 1986). Exposure durations resulting in critical damage to root tissue decreases as RT increases. Root browning in hydroponically-grown chrysanthemum (*Dendranthema* × *grandiflorum* 'Paragon') occurred after 25 min with a 45°C RT (Macdonald 1991). The temperature at which 50% of cell solutes are lost is used as an indicator of acute damage to tissues (LT_{50}; Levitt 1980). For many woody plant species, sigmoidal increases in root electrolyte leakage occur with increasing RT with critical thresholds occurring between 45 and 57°C at 20-30 min of exposure (Ingram *et al.* 1986; Donovan *et al.* 1990; Martin *et al.* 1991; Sibley *et al.* 1999). The short exposure times of 20-30 min used in these past studies reflect the rapidity at which root damage occurs at high temperatures. However, research on short periods of exposure that represent daily diurnal solar exposure heating, as well as air temperature heating, is lacking on both woody and herbaceous plants. Thresholds may vary between varieties of species, but few comparative studies have been undertaken to explore this. A study of red maple (*Acer rubrum* and *A. x fremannii*) varieties from different sites of origin

determined that critical root damage thresholds fell between 52 and 53.5°C after 30 min of exposure (Sibley *et al.* 1999).

The destabilization of cell membranes by short RT exposure is partially a product of reactive oxygen species (ROS) accumulation due to increased respiration (Breusegem & Dat 2006). Peroxidation of lipids reduces cell membrane stability and can result in intercellular solute loss. Heat-sensitive creeping bentgrass (*A. stolonifera* 'L-93') root exposure to 37°C for 19 d resulted in membrane leakage of up to 80% of the amino acids present in tissues (Lyons *et al.* 2007). A 12-hour exposure of *Jatropha curcas* roots to 42°C resulted in higher ROS concentrations and membrane peroxidation than in shoots grown at 27°C (Silva *et al.* 2017). Two heat-sensitive cucurbit species (*Cucurbita ficifolia* and *C. maxima*) had increased H_2O_2 and malondialdehyde (MDA; a byproduct of lipid peroxidation) root tissue concentrations when subjected to RT of 34°C for 7 d compared to plants grown with 14 or 24°C RT (Zhang *et al.* 2007). A reduction in fatty acid saturation and increased MDA concentration in bentgrass (*A. stolonifera* 'Penncross') roots and leaves corresponded to reductions in P_n, associated with premature senescence of leaf tissues associated with ROS accumulation at RT of 35°C (Liu & Huang 2004).

High RT can also potentially decrease root water uptake. Increased cell membrane permeability and reduced aquaporin concentration in broccoli (*Brassica oleracea var. italica*) roots were observed when RT increased from 35 to 45°C (Iglesias-Acosta *et al.* 2010). Transfer of aeroponically-grown peppers (*Capsicum anuum*) from 20°C RT to diurnally-fluctuating RT of 25 and 40°C for 23 d decreased root hydraulic conductivity by 80% (Dodd *et al.* 2000). Uptake and internal transport of potassium (K^+),

an ion important to the maintenance of osmotic exchange in root tissues, was limited in olive (*Olea europea* 'Arbequina') seedlings grown in a 37°C hydroponic solution for 33 days and may have contributed to reduced root and shoot dry matter gain (Benlloch-González *et al.* 2017). Similar reductions in root tissue K+ concentration were observed in bentgrass (*A. stolonifera* v. 'L93' and 'Penncross') grown at 35°C RT with both 20 and 35°C air temperatures, and were accompanied by reduced P_n and g_s relative to plants with 20°C RT (Huang & Xu 2000). Reduced root hydraulic conductivity impacts above ground cell water potential, g_s, and subsequently P_n. For instance, reduced leaf relative water content of butterhead lettuce (*Lactuca sativa* 'Palma') grown in an aeroponic system with fluctuating ambient RT of 23 to 40°C was associated with lower g_s and net photosynthetic CO_2 assimilation (A_{sat}) compared to plants maintained at 20°C RT (He *et al.* 2001). Twenty-eight percent reductions in leaf water potential and reduced g_s were observed in thermotolerant bunchgrass (*D. lanuginosum*) grown at 42°C RT, and diminished soil water uptake compared to plants grown at RTs of 30 or 36°C (Germino & Wraith 2012).

The effects of high RT-induced ABA synthesis on g_s and associated impacts on P_n have not been extensively studied. In six Cucurbit (*Cucumis*) species, reductions in g_s and net P_n were associated with increased ABA levels in leaf tissues of plants grown at RT above or below temperature optima (Zhang *et al.* 2008). Reduced g_s and P_n in cucumber (*C. sativus* 'Suyo') leaves accompanied increased leaf ABA concentrations when plants were exposed to RT of 38°C for ten days compared to plants grown at 30°C (Nada *et al.* 2003). Reduced P_n in rice (*Oryza sativa*) was associated with root-generated ABA and decreased g_s (Arai-Sanoh *et al.* 2010a). However, reductions in g_s and P_n of

peppers (*Capsicum anuum* 'Jin Jao No. 3'*)* were not linked to increased tissue ABA, cytokinins, or altered xylem pH (Dodd *et al.* 2000). This suggested that varying physiological factors and/or signals play a role in RT-induced changes in P_n in herbaceous plant species.

The role of high RT in the alteration of photosynthetic efficiency (F_v/F_m) is uncertain. At high air temperatures, F_v/F_m is reduced due to heat-induced ROS accumulation and damage to photosystem II. Reductions in F_v/F_m were observed in tomato plants exposed to high air temperatures (Camejo *et al.* 2005; Zhou, Yu, *et al.* 2015), though associations with high RT remain scarce. Creeping bentgrass (*A. scabra* and *A. stolonifera* 'L93' and 'Penncross') P_n and F_v/F_m were reduced at a 37°C RT compared to plants grown at 20°C, though the degree of reduction varied between species (Rachmilevitch *et al.* 2006). Lower F_v/F_m was also observed in the leaves of butterhead lettuce (*L. sativa* 'Palma') exposed to fluctuating 23 to 40°C RT compared to plants grown at consistent 20°C RT (He *et al.* 2001). However, the F_v/F_m of cucumbers (*C. sativus* 'Suyo') was not lower at RT 38°C than at RT of 30°C, despite observed reductions in leaf carbon exchange rates (Nada *et al.* 2003).

Physiological Mechanisms of Root Thermotolerance

The ability of plants to withstand single or repeated high RT events, through mechanisms such as carbon partitioning or antioxidant production, is critical to continued survival and growth in a world with increased climate change. In natural settings, plant plasticity to high RT is associated with stress-alleviation pathways such as antioxidant production by thermotolerant species. High RT can limit growth of both

thermotolerant and sensitive plants, but more so for heat-sensitive species. For example, Rachmilevitch *et al.* (2006) found the thermotolerant bentgrass species *A. scabra* maintained a higher root relative growth rate compared to the heat-sensitive *A. stolonifera* 'Penncross' and 'L-93'at 37°C RT over a 28d period. The up-regulation of genes underlying root heat tolerance was highest in a heat-tolerant variety of rice (*Oryza sativa*), 'N22', over a less heat tolerant variety, 'Vandana', when subjected to short and long exposures to 42/36°C air temperatures (day/night) (Sailaja *et al.* 2014). Tolerance of 38°C RT amongst foxtail millet (*Setaria italica*) varieties was associated with lower impacts of RT on root growth (dry mass, volume, diameter) and dark respiration (Aidoo *et al.* 2016).

Root respiratory acclimation and carbon partitioning reduce long-term impacts of high RT. Respiratory acclimation potential of the thermotolerant bunchgrass *A. scabra* was greater than that of the related heat-sensitive species *A. stolonifera* 'Penncross' during both short-term (1 hr) and long-term (7-28 d) root heat events (Rachmilevitch *et al.* 2008). The ability of *A. scabra* to regulate respiration across varying high RT durations may represent two alternative scenarios for temperature acclimation by plant tissues. In one scenario, existing tissues alter respiration rates in response to elevated temperatures; in the other, new tissues produced during heat stress events are conditioned to exhibit higher respiratory thresholds (Atkin & Tjoelker 2003). The adaptation of new tissues to better withstand high RT can also extend to increased membrane integrity. For example, in the cacti species *Nopalea cochenillifera* and *Opuntia robusta*, increases in diurnal air temperatures by 20°C can result in a 3.4°C-higher LT_{50} value in root cortical cells (Nobel & Zutta 2008).

Development of new tissues with greater thermotolerance depends on carbon allocation to new growth rather than maintenance of existing metabolic activities. The thermotolerant bentgrass species *A. scabra* more efficiently allocates ^{14}C into new proteins and lipids than *A. stolonifera* 'Penncross' at RT of 37°C, though growth remains limited compared to plants grown at 20°C (Rachmilevitch *et al.* 2015). New tissues acclimate more quickly than old tissues to elevated growing temperatures across both fast- and slow-growing plant species, though overall respiratory acclimation is not correlated with relative growth rate (Loveys *et al.* 2003). Limited allocation of resources to new tissues under high RT may also impact their metabolic potential. The thermotolerant grass *Andropogon gerardii* (C4) increases total root mass when growing at air temperatures of 35-40°C, but the respiration, nutrient uptake, and exudation per gram of root mass was reduced (Mainali *et al.* 2014).

Root tissue acclimation may depend on shifts in respiration from the cytochrome (COX) to the alternative oxidase (AOX) pathway. Increased use of the AOX pathway under stress conditions was associated with reduced ROS production and lipid peroxidation (Keunen *et al.* 2013). Higher thermotolerance in *A. scabra* over *A. stolonifera* was attributed to the ability to use the AOX pathway when heat stressed (Rachmilevitch, Lambers, *et al.* 2006). The maintenance of ATP activity in cucumber (*C. sativus* 'Sharp I') roots exposed to 38°C RT was also associated with a switch to this alternative respiration pathway (Du & Tachibana 1994a). However, Q_{10} values associated with the AOX pathway do not differ enough from the COX pathway to suggest a greater efficiency in energy use under stress (Atkin & Tjoelker 2003). The AOX pathway may

help reduce buildup of stress-induced ROS, but it may not be able to fully compensate for heavy accumulations under stress conditions (Zhang *et al.* 2012).

Increased antioxidant production in response to high RT buffers ROS accumulation and can contribute to heat tolerance. Greater catalase production and lower reductions in superoxidative dismutase and peroxidase levels in root tissues of thermotolerant bentgrass (*A. scabra* 'NTAS') were observed relative to the related species *A. stolonifera* 'Penncross' when both were exposed to 35/30°C air temperatures for 24d (Xu *et al.* 2015). Increases in ROS (superoxide and hydrogen peroxide) concentration in the roots of *A. scabra* 'NTAS' were consequently lower than those of *A. stolonifera* 'Penncross'. Differing heat tolerance in two varieties of Kentucky bluegrass (*Poa pratensis* 'Midnight' and 'Brilliant') was partially a product of reduced root electrolyte leakage associated with antioxidant defense-linked protein upregulation in roots (Zhang & Du 2016). Higher levels of antioxidant production by the rootstocks of thermotolerant species can alleviate high RT on grafted scion shoot tissues of temperature-sensitive species. Cucumber (*Cucumis sativus*) grafted onto thermotolerant luffa gourd (*Luffa aegyptiaca*) rootstocks had lower leaf damage due to oxidation at high air temperatures than cucumber-cucumber grafts; cucumber roots are less heat tolerant than luffa (Li *et al.* 2014, 2016).

Heritability of both below and aboveground heat tolerance characteristics remains challenging to identify and promote in the selection of new varieties, having received limited attention from plant breeders (Wahid *et al.* 2007). Defensive antioxidant and ROS production of the hybrid Cucurbit 'Maxchata' was in-between that of its parent species *Cucurbita moschata* and *C. maxima* in response to elevated diurnal air

temperatures (Ara *et al.* 2013). However, the levels of specific antioxidants such as superoxide dismutase (SOD) and catalase (CAT) produced in 'Maxchata' paralleled that of either one or the other parent.

Plants exposed to supraoptimal RT can recover. For example, g_s, A_{sat}, and midday leaf relative water content of butterhead lettuce plants (*L. sativa* 'Palma') grown at fluctuating ambient 23-40°C RT returned to levels similar to plants maintained at 20°C RT when the stressed plants were transferred to 20°C RT (He *et al.* 2001). However, recovery by these plants took up to 10 d after transfer. Severity of tissue damage and extent of carbohydrate resource depletion following high RT exposure may slow recovery time and the resumption of healthy growth. Degradation of cellular proteins important to the uptake of nutrients at RT of 40°C prolonged the recovery period of tomatoes (*Solanum lycopersicum* 'Bigboy') after treatment (Giri *et al.* 2017). Root elongation of sorghum (*Sorghum bicolor* 'Mairo' and 'Saitama') grown at 40°C RTs and returned to 25°C RT was less than plants grown at 25°C (Pardales *et al.* 1991). Diurnal variation in RT between 40°C and 25°C reduced the impact of high RT treatments on root growth compared to constant 40°C RT, suggesting intermittent periods of lower RTs can reduce the negative impacts of high RT. Given that diurnal variation in temperatures is absent from the bulk of previous long-term RT studies, the effects of exposure may be over-estimated.

Potential Microbial Alleviation

Plant sensitivity to high RT may be ameliorated by using microbial inoculants. The presence of plant growth promoting microbes has been associated with

improved plant growth under abiotic and biotic stress conditions through a variety of factors including regulation of hormones, improved nutrient and water uptake, and competition with pathogens (Backer *et al.* 2018). Interactions between plants and microbes may vary between species, but can be multi-faceted. For example, treatment of *Arabidopsis thaliana* ('Columbia') seedlings with *Bacillus licheniformis* 'CH102' improved resistance to heat and drought stress while also increasing the activation of disease resistance genes (Sukkasem *et al.* 2018).

Stimulation of antioxidant activity by microbes is one mechanism for improved plant tolerance of heat stress. Peroxidase, superoxide dismutase, and catalase activity in leaves and roots of *Septoglomus constrictum*-inoculated tomatoes (*Solanum lycopersicum* 'Moneymaker') was higher under heat and drought stress conditions than in un-inoculated plants, and corresponded with reduced MDA and H_2O_2 content (Duc *et al.* 2018). Similarly, improved salinity tolerance by tomatoes (var. 'Zhongzha105') inoculated with *Glomus mosseae* was associated with elevated levels of antioxidants in the leaves and accompany reductions in MDA content (Abdel Latef & Chaoxing 2011). Reductions in MDA content and root membrane permeability were also associated with *Glomus etunicatum* inoculation of maize (*Zea mays var.* 'Zhengdan 958') grown at both supra- and sub-optimal air temperatures ranging between 5-40°C (Zhu *et al.* 2010).

Root-associated microbes can also reduce stress-induced hormone signaling in plants. Production of indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC)-deaminase by plant growth promoting bacteria (PGPBs) is associated with increased halotolerance and drought resistance in crops. Inoculation of salt-stressed canola (*Brassica napus*) seedlings with ACC-deaminase producing *Enterobacter cloacae*

'HSNJ4' reduced tissue concentrations of stress-induced ethylene and also increased tissue antioxidant activity (Li *et al.* 2017). ACC-deaminase associated reductions in tissue ethylene content also occurred in salt-stressed container-grown wheat (*Triticum aestivum*) as a consequence of inoculation with *Pseudomonas putida* 'N21', *Pseudomonas aeruginosa* 'N39', or *Serratia proteamaculans* 'M35' (Zahir *et al.* 2009). Inoculation of salt-stressed tomato (*S. lycopersicum* 'F144') seedlings with ACC-deaminase-producing *Achromobacter piechaudii* 'ARV8' reduced ethylene synthesis and increased fresh and dry weight (Mayak *et al.* 2004).

While good evidence exists for the ability of microbial inoculants to reduce high RT stress in plants, most studies have only focused on plant responses to elevated air temperatures. The effectiveness of microbial inoculants under high RT may depend on their ability to withstand the high temperatures concurrently. For example, lower root colonization rates by *Glomus intraradices* compared to another *Glomus sp.* 'AZ112' in peppers (*C. anuum*) was observed at 32-38°C (Martin & Stutz 2004). Variation in arbuscular mycorrhizal fungi (AMF) community survival in high temperature soils is dictated by temperature extremes at their sites of origin (Zhou, White, *et al.* 2015). Therefore, identifying naturally thermotolerant microbial strains will be important for successful applications in plant production when attempting to alleviate negative high RT effects on plant growth and crop yield. Recent examples of the effectiveness of heat-tolerant microbes include the survival of bunchgrass (*Dichanthelium lanuginosum*) grown at RT of 50°C; *D. lanuginosum* was dependent on the presence of *Curvularia sp.* fungus isolated from geothermal soils of Yellowstone National Park (Redman 2002). Subsequent treatment of tomato (*S. lycopersicum v.* 'Rutgers') with *C. protuberata*

improved survival rate of seedlings exposed to 65°C RT for 10hr/d across 14d (Luis M. Márquez 2007). Treatment of rice (*Oryza sativa*) seedlings with a thermotolerant strain of *C. crepinii* ('G1-29'), improved survival over extended growth periods at RT of 50°C (Zhou et al., 2015). In another study, *Pseudomonas sp.* 'AKM-P6' isolated from the roots of pigeon peas (*Cajanus cajan*) grown in a warm, semi-arid environment increased the root and shoot biomass of sorghum (*Sorghum bicolor* 'CSV-15') seedlings grown at 47-50°C/30-33°C (day/night) temperatures and extended their survival ten days longer than un-inoculated plants under the same conditions (Ali *et al.* 2009).

Concluding Remarks

Production of herbaceous plants in sun-exposed containers during warm summer months or in warm climates commonly expose roots to high RT that decrease growth and negatively impact plant functioning. These high RTs may impact above-media growth by increasing respiratory energy demands and decreasing P_n. Direct damage to root systems through membrane destabilization and ROS accumulation can reduce hydraulic conductivity and trigger stress-induced synthesis of ABA and reductions in g_s that negatively impact P_n. Naturally thermotolerant plants have the ability to mitigate some of these effects by managing ROS accumulation through antioxidant production and the efficient partitioning of carbohydrates into new, heat-adapted tissues. Microbial inoculants may help to buffer heat-sensitive plants to high RT through similar pathways and deserve additional attention as an alternative, biological means for the alleviation of negative high RT effects on roots, shoots and yield.

Chapter 2

Acute high root temperature impacts tomato (*Solanum lycopersicum*) root respiration and electrolyte leakage

Growing plants in containerized systems can result in high root temperatures (HRT) as containers, media and roots are above the ground and exposed to air and sunlight; temperatures over 50°C are commonplace. Long-term HRT can damage root tissues directly, and plant health generally, by reducing the plant carbohydrate status. Short-term HRT thresholds that cause direct damage and root death have not been determined for many herbaceous plant species. We evaluated how short-term HRT impacted root respiration and electrolyte leakage of eight tomato varieties characterized as 'heat-tolerant' or 'sensitive' based upon aboveground characteristics. Respiration rates and electrolyte leakage of excised root masses heated to 48 to 62°C were quantified. Root respiration rates increased from 21.6 μmol hr^{-1} g^{-1} at 48°C to 26.9 μmol hr^{-1} g^{-1} at 48-51°C, and then decreased to 0 μmol hr^{-1} g^{-1} at 57°C. Varieties did not differ in responses to root temperature. However, overall 'Solar Fire' (heat-tolerant) had a higher respiration rate across temperatures. Root temperature and variety interacted to impact electrolyte leakage as a proportion of total electrolyte leakage. Proportional electrolyte leakage of 'Nacgarlang' (heat-tolerant) increased from 0.54 at 50°C to 0.87 at 52°C and reached a maximum of 1.09 at 54°C, whereas 'Solar Fire' root electrolyte leakage increased incrementally from 0.27 at 50°C to 0.60 at 54°C, but did not reach the point of maximum leakage until 62°C. Our results suggested critical physical and metabolic damage

occurs to tomato roots at ≥50°C. Additionally, root respiration and electrolyte leakage data provide insight into temperatures when roots are active and/or maintain physical integrity. The lack of strong differences between critical respiratory and electrolyte leakage temperature thresholds amongst varieties suggested root physiological responses to HRT do not correspond with aboveground heat tolerance.

Introduction

Containerized crop production of herbaceous plants is commonplace in the ornamental and vegetable production industries. Solar heating and conduction can increase medium temperatures above air temperatures to levels that may be detrimental to root growth. For instance, media temperatures as high 55-57°C were observed in sun-exposed containers in Minnesota (Guenthner, personal observation). Media temperatures of sun-exposed containers in California reached 52°C (Lyles *et al.* 1992) or above (J. Erwin, personal observation).

Roots are more sensitive to supra-optimal temperatures than aboveground plant tissues (He *et al.* 2001; Tahir *et al.* 2008; Sailaja *et al.* 2014; Giri *et al.* 2017). Aside from sensitivity to high root temperature (HRT), above and below ground biomass are reduced by HRT (Du & Tachibana 1994a; Klock *et al.* 1997; Liu & Huang 2000; Benlloch-González *et al.* 2017). Reduced biomass due to HRT results from both acute and chronic temperature effects on photosynthesis and respiration. Acute high temperatures damage root tissues through membrane destabilization and lipid peroxidation (Ingram *et al.* 2015). Membrane destabilization is often quantified by measuring electrolyte leakage (EL) from roots in solution by monitoring changes in

electroconductivity. Increases in EL with rising temperature follow a trend of sigmoidal change, and temperature thresholds for critical damage are often defined as the point where EL is 50% greater than baseline levels in solution at non-stress RT (Ingram *et al.* 1986; Donovan *et al.* 1990; Martin *et al.* 1991; Sibley *et al.* 1999). Exposure times associated with these critical damage thresholds are less than 1hr at RT above 45°C for some woody plant species (Ingram *et al.* 1986; Donovan *et al.* 1990; Sibley *et al.* 1999).

Extended exposure of roots to high temperature can increase respiration and alter carbohydrate allocation (Hurewitz & Janes 1983; Du & Tachibana 1994b; Klock *et al.* 1997; Huang & Xu 2000; Rachmilevitch *et al.* 2015). Increased carbohydrate allocation to roots at HRT (and respiration) can negatively impact plant growth (Du & Tachibana 1994b). Root respiration rates generally double for every 10°C increase in temperature (known as the Q_{10}) and are often used as indicators of metabolic activity (Atkin & Tjoelker 2003); respiration rate has not been used as an indicator of high temperature thresholds for root metabolic activity.

Variation in temperature thresholds for root damage and death amongst plant species and varieties remains poorly explored. Critical thresholds for root electrolyte leakage of nine varieties of red maple (*Acer rubrum* and *A. x freemanii*) were between 52 and 53.5°C with a 30 min exposure (Sibley *et al.* 1999). Tolerance of HRT varies among creeping bentgrass species (*Agrostis sp.*) and is associated with differences in antioxidant buildup, respiratory activity, and carbohydrate allocation (Rachmilevitch, Lambers, *et al.* 2006; Rachmilevitch *et al.* 2015). However, previous studies of supraoptimal root temperatures effects on herbaceous plants focused on root and whole plant responses to extended, sub-lethal HRT (Du & Tachibana 1994a; Klock *et al.* 1997; Huang & Xu 2000;

Lyons *et al.* 2007; Arai-Sanoh *et al.* 2010a). An understanding of root temperature thresholds for acute short-term high temperature exposure periods is lacking for the bulk of herbaceous plants. This is of special significance in that HRT in containers can be frequent, short-term and isolated to mid- to late afternoon.

The study presented here explores root respiration and electrolyte leakage responses of eight tomato (*Solanum lycopersicum*) varieties that vary in previous characterizations of aboveground heat tolerance (Rudich *et al.* 1977; Abdul-Baki 1991; Barten *et al.* 1992; Camejo *et al.* 2005; Scott *et al.* 2006; Kamel *et al.* 2010; Bita *et al.* 2011; Zhou, Yu, *et al.* 2015). Specifically, we sought to 1) evaluate root respiration and electrolyte leakage responses to acute, HRTs, 2) determine whether above-media heat-tolerance was associated with root respiration and electrolyte leakage responses to HRT and 3) determine whether evaluation of root responses to HRT was better undertaken using respiration or electrolyte leakage techniques. We anticipated that root respiration and electrolyte leakage responses to HRT would not be associated with previous characterizations of heat tolerance or sensitivity amongst varieties.

Materials and Methods

Experiment I: Root Respiration

Eight tomato varieties identified as heat-tolerant or -intolerant based on aboveground characteristics such as fruit set and photochemical efficiency were selected for this study (see Table 1). The tomato varieties ‘LA1994’, ‘Nacgarlang’, ‘Saladette’, ‘Solar Set’ and ‘Solar Fire’ represented ‘heat-tolerant’ varieties. ‘Amana Orange’, ‘Moskvich OG’ and ‘Campbell 28’ represented ‘heat-sensitive’ varieties (Table 1). Seed

of ‘Amana Orange’ was obtained from Tomato Grower’s Supply (Fort Meyers, FL), seed of ‘Moskvich OG’ was obtained from Johnny’s Selected Seeds (Winslow, ME), ‘Solar Fire’ and ‘Solar Set’ were obtained from the University of Florida (Gulf Coast Research and Education Center; Balm, FL), and seed of ‘Saladette’, ‘Campbell 28’, ‘LA1994’ and ‘Nacgarlang’ were obtained from the Tomato Genetics Resource Center (Davis, CA).

Seed were sown into 50-cell trays (one seed per cell; cell vol. 75 mL) (TO Plastics, Clearwater MN) in a soilless media (SunGro SS#8-F2; Agawam, MA) and were lightly covered with vermiculite (3mm). Sown seed were placed in a mist greenhouse maintained at 26.1 ± 1.7°C and 22 ± 2.1°C day/night temperatures under natural daylight conditions with 100 ± 20 µmol m^{-2} s^{-1} supplemental light (when light levels were below 420 µmol m^{-2} s^{-1}) for 11 d. Trays were initially watered manually and were watered thereafter with periodic automated mist (cycling 8-24 sec duration every 15 min between 0615 and 2215 HR, daily). Following germination (emergence of the radicle) and after two true leaves unfolded, seedlings were transferred to a greenhouse under natural daylight conditions (22.7 ± 8.7 mol m^{-2} d^{-1}) and day/night temperatures of 28.5 ± 4.4°C and 22.5 ± 2.4°C, respectively. Plants were watered as needed to maintain a moist media and fertilized through the irrigation water with Peters Excel CalMag 15-5-15 (ICL Specialty Fertilizers; Summerville, SC) at a concentration of 250 ppm N. Plants were spaced in additional 50 cell trays after approximately 4 wks. to facilitate uninhibited growth.

Once roots covered 75% or more of the external surface of a rooted cell, plants were transferred to an environmental growth chamber (Environmental Growth Chambers; Chagrin Falls, OH) with a 12-hr day/night photoperiod (325 µmol m^{-2} s^{-1}) provided by

fluorescent (75% wattage) and incandescent lamps (25% total wattage). Plants had 6 to 12 true leaves unfolded at that time, depending on the variety. Chamber relative humidity was set to 40%. Plants were watered periodically with clear irrigation water to maintain moist media.

Above-media tissue (stem and leaves) were removed from below-media tissue (roots) after one to three days. Isolated root masses were placed in a waterproof 50 cell tray approximately 80% submerged (from the bottom) in a water bath heated to different temperatures (48, 51, 54, and 57ºC). These temperatures were selected based on preliminary research suggesting upper thresholds for critical root damage were within this heat range and media temperature data collected from sun-exposed plastic containers. Temperature treatments were performed in a system that incorporated plastic storage bin water baths (vol. 15 L each; Rubbermaid, Atlanta GA) that contained water that was heated to desired temperatures using an immersion heater and circulator (Gourmia GSV-140; Brooklyn, NY). A digital temperature probe (Quartz Digi-Thermo, Traceable Products; Webster TX) was inserted in one root mass 1 cm from the edge of the cell and monitored to determine when roots reached the desired temperatures (± 0.2ºC). Once roots reached the desired treatment temperature, edge root mass temperature was recorded when they were removed from the plastic cell. Root masses were immediately transferred to the bottom of a pre-heated glass jar (240 mL) with roots oriented upwards for maximized root exposure to air. Jars were sealed with airtight metal lid and were placed in a water bath likewise pre-heated using an immersion heater set to temperatures previously determined to maintain inner-jar air temperatures equal to those of the desired temperature treatment. 3-6 root masses of each variety were tested at a time.

A SO-#110 galvanic oxygen cell sensor (Apogee Instruments, Logan UT) was mounted vertically in the center of each metal jar lid with the sensor head suspended 0.5 cm into the jar. Sensors were sealed with manufacturer-supplied rubber O-rings and vacuum grease to prevent gas leakage from jars. These sensors measured absolute air oxygen (hereafter O_2) concentration in the jar, but were calibrated to report partial pressure (kPa; relative concentration of O_2). Sensors were paired with a CR1000 Datalogger and accompanying LoggerNet program (Campbell Scientific; Logan UT) to collect and record data. Sensors also contained active internal thermistor heaters to reduce condensation buildup on the membrane inside the sensor head. Sensors were calibrated daily to an ambient local partial pressure of approximately 20.69 kPa. 20-30 mL of Drierite (WA Hammond Drierite Co. Ltd.; Xenia, OH) desiccant was added to the bottom of sample jars to reduce condensate; sensor output declined rapidly when condensation formed on the sensor head/membrane. In a separate assessment, the atmospheric effects of Drierite did not impact measurement of O_2 concentration within jars when compared to jars that did not contain Drierite. Throughout the sampling period, the water-bath-sample-jar setup was covered with a blanket to limit air temperature fluctuations and the cooling of jar lids that encouraged condensation. Between measurements, a small desk fan was used to blow air across sensors for approximately 1-1.5 hr to reduce buildup of condensation that might impact O_2 readings on internal sensor membranes. Un-rooted 50 cell plugs containing the same growing media maintained in the same production trays as rooted cells were also tested to identify any incidental atmospheric effects or respiratory effects from biological activity in the media other than roots.

Partial pressure data were recorded by the datalogger (CR1000; Campbell Scientific, Logan UT) every 10 sec over 1.25-1.5 hr. Data from the first hour of data after jars were sealed was discarded as this period included atmospheric adjustment from opening and closing jars. Data from the remaining 15 to 30 min decreased linearly as O_2 levels decreased as respiration occurred.

Data were converted from partial pressure (kPa) to mol O_2 prior to analysis using the ideal gas law:

$$PV/RT = n$$

Where P = recorded partial pressure in the sample jar, V = gas volume in the sample jar with root mass and Drierite (approx. 0.135 L), T = treatment temperature (in °K), R = gas constant (8.31446), and n = calculated mol O_2. Resulting O_2 concentration data for each sample was regressed over time using linear regression and the slope of the regression function was used as the estimated rate of O_2 reduction over time. Average coefficients pooled from non-rooted cells in jars measured with and without Drierite were subtracted from corresponding rooted-sample temperature treatment slopes to correct for potential atmospheric and microbial contributions to changes in O_2 concentration over time. These adjusted respiration rates (slopes) for each root mass were converted from mol O_2 sec^{-1} to μmol O_2 hr^{-1} in convention with previously reported data and divided by corresponding fresh and dry mass values (g^{-1}) to normalize data across fresh and dry weight.

Following the O_2 measurement period, roots were washed free of media, patted dry on paper towels and weighed to obtain root fresh mass. Roots were placed in a drying

oven (Hotpack Corp., Philadelphia PA) at 65ºC for three or more days, after which they were weighed again to determine dry mass.

This experiment was organized in a completely randomized factorial statistical design in a factorial arrangement, where 3-6 root masses (replicates) of each variety were evaluated for O_2 consumption (respiration) rates at each treatment temperature. Respiration rates of root masses normalized on a fresh and dry weight basis were analyzed using one-way ANOVA with root temperature and tomato variety as the main effects. All data was processed and analyzed using SPSS Statistics v. 24 (IBM Co.; Armonk NY). Outliers were identified using the SPSS 'Explore' function and were removed when identified (data which lay outside the interquartile range by three times its inner range). Tukey's$_{HSD}$ was used for mean separation in all cases where significant differences were found.

Experiment II: Root Electrolyte Leakage

Tomato varieties were selected based upon the same criteria of heat tolerance or sensitivity as described above, and grown under the same greenhouse conditions in 72 cell production trays (cell vol. 60 mL). After one to three days, plants of each variety were destructively sampled by removing above-media tissue (stem and leaves) from below-media tissue (roots). Root masses were washed and lightly patted with paper towels to remove excess water and obtain fresh mass values. Root masses were placed in sealed 50 mL vials to prevent desiccation due to additional air-drying while additional samples were prepared. At the time of temperature treatment, 30 mL of nanopure water (electroconductivity [EC] 0.00 ± 0.01 ms^{-1} cm^{-3}) was added to each sample vial and root

masses were fully submerged within. Sample vials were sealed and 75% submerged in a water bath heated to the desired treatment temperature by an immersion heater and circulator (Gourmia GSV140; Brooklyn, NY). A Quartz Digi-Thermo temperature probe (Traceable Products; Webster TX) was inserted into an identical sample jar containing 30 mL of nanopure water and monitored to determine when the desired temperature point had been reached within root sample jars. Treatment temperatures were 50, 52, 54, 56, 58, 60, and 62°C (± 0.03°C), based upon previous research suggesting critical damage thresholds for roots fell within this range. Once the desired water temperature was reached within the vial, samples were held at that temperature for 30 min. Following this exposure period, sample vials were removed from the water bath and cooled at room temperature (23 ± 0.5°C) for 15 min. Electrolyte solution was decanted through a fine mesh strainer from each sample into separate vials, all of which were refrigerated at 2°C until measurements were performed. Root masses were removed from sample vials after solution decanting and subsequently dried in an oven (Hotpack Corp., Philadelphia PA) at 65°C for three days or more prior to measurement of dry weight.

Root masses heated to 62°C were additionally brought to a boil to determine maximal electrolyte leakage from damaged root tissues. For this temperature treatment, the same procedure as above was followed for treatment of roots at 62°C. However, following treatment at 62°C, sample solutions were decanted, cooled to room temperature, and electroconductivity readings were taken. Sample solutions were added back to corresponding root samples and brought to boiling temperature in an electric microwave (1300 W; Panasonic, Kadoma JP). The same 15 min cooling period at room temperature and subsequent decanting procedure was followed.

Measurement of solution electroconductivity, as a representation of the root EL of each sample, was done using an YSI Model 35 Conductance Meter (YSI Inc.; Yellow Springs, OH) within 1.5 wk of sampling. Samples were removed from refrigeration one day prior to measurement and allowed to equilibrate to room temperature. Measurements were taken at a solution temperature of 23.0°C (± 0.03°C) and the conductivity meter was standardized between readings using nanopure water (0.00 ± 0.01 ms^{-1} cm^{-3}) and HI 70422 standard solution (Hanna Instruments, Woonsocket, RI). Microbial growth was noted in a number of stored sample vials prior to measurement, but subsequent testing of a subgroup of these samples before and after boiling (to re-suspend trapped solutes) demonstrated no effect on sample solution conductivity.

This experiment was organized using a completely randomized factorial design, with 4-5 root masses (replicates) from each variety, that were sampled for each temperature treatment. EL data normalized on a fresh and dry weight basis was analyzed via one-way ANOVA with temperature and variety as the main effects. Where interactions occurred, secondary analysis via two-way ANOVA was performed. Tukey's$_{HSD}$ was used for mean separation whenever significant effects were found. Following analysis of absolute EL values, further analysis was performed to assess EL of samples as a proportion of values obtained through the maximal damage (boiling temperature) treatments. Mean EL values (normalized for fresh and dry mass) were first determined for each tomato variety at boiling temperature and sample values at other temperatures were individually divided by corresponding varietal maximum mean values to obtain proportion total EL values. Proportional EL values were Arcsine transformed and analyzed via one-way ANOVA with Tukey's$_{HSD}$ used for mean separation. Follow-

up analysis via two-way ANOVA was performed where interactions between main effects occurred. All statistical analyses was performed using SPSS v. 24 (IBM Co.; Armonk, NY). Throughout data analysis, identification and elimination of outliers was performed using the 'Explore' function of the SPSS statistical software.

Results

Experiment I: Root Respiration

Respiration rates normalized per g^{-1} fresh weight were affected by temperature ($p \leq 0.001$) and variety ($p \leq 0.001$) independently (Table 2.2). Respiration rates per g^{-1} fresh weight increased from 1.83 μmol hr^{-1} g^{-1} at 48°C to 2.30 μmol hr^{-1} g^{-1} at 51°C, and decreased incrementally to 1.39 μmol hr^{-1} g^{-1} at 54°C and -0.47 μmol hr^{-1} g^{-1} at 57°C. Respiration rate per g^{-1} fresh weight of 'Solar Fire' across RT (1.97 μmol hr^{-1} g^{-1}) was higher than all other varieties with the exception of 'Solar Set' (1.51 μmol hr^{-1} g^{-1}) and 'Saladette' (1.52 μmol hr^{-1} g^{-1}).

Respiration rates normalized per g^{-1} dry weight were affected by temperature ($p \leq 0.001$) and variety ($p \leq 0.01$) independently (Table 2.2). Root respiration rates per g^{-1} dry weight across varieties increased from 21.58 μmol hr^{-1} g^{-1} at 48°C to 26.93 μmol hr^{-1} g^{-1} at 51°C, and decreased to -0.52 μmol hr^{-1} g^{-1} as temperature further increased to 57°C (Fig. 2.1). 'Solar Fire' had a greater respiration rate across temperatures (21.81 μmol hr^{-1} g^{-1}) than any other variety except 'Solar Set' (Fig. 2.2). However, 'Solar Set' did not differ from any other varieties in the study with a respiration rate across temperatures of 16.98 μmol hr^{-1} g^{-1} (Fig. 2.2).

Experiment 2: Root Electrolyte Leakage

Root temperature and variety interacted to affect EL normalized per g^{-1} root fresh weight and dry weight (Table 2.3, $p \leq 0.001$ in all cases). As fresh weight can be impacted by root water content, electrolyte leakage responses on a g^{-1} dry weight basis will be presented and discussed for the remainder of the paper. EL per g^{-1} dry weight increased across varieties as temperature increased from 50 to 54°C. Varieties differed in their EL per g^{-1} dry weight response at different temperatures. For example, 'Nacgarlang' EL increased from 3.48 ms cm^{-3} g^{-1} at 52°C to 4.73 ms cm^{-3} g^{-1} at 54°C, but EL values at higher temperatures did not differ (Table 2.4). Similar EL trends were observed for 'Solar Set', 'Saladette', 'Moskvich OG', 'Amana Orange' and 'LA1994', though EL peaked at 58°C for 'Amana Orange' and 'Campbell 28' (Table 2.4). The EL of 'Solar Fire' increased from 3.25 ms cm^{-3} g^{-1} at 52°C to 4.44 ms cm^{-3} g^{-1} at 54°C and plateaued before increasing again to its maximum value of 7.57 ms cm^{-3} g^{-1} at 62°C (Table 2.4).

Proportional EL per g^{-1} dry weight was impacted by an interaction between RT and variety ($p \leq 0.001$), generally increasing across varieties with increasing RT (Table 2.5). The lowest threshold for complete root damage (100% proportional EL) was observed for 'LA1994' at 52°C, whereas 'Moskvich OG', 'Nacgarlang', and 'Saladette' had complete damage at 54°C and 'Amana Orange' reached its point of complete root death at 58°C (Table 2.6). Two other varieties, 'Campbell 28' and 'Solar Set' did not reach the complete damage threshold by 62°C, though both of these varieties plateaued in their proportional EL by 54°C (Table 2.6). 'Solar Fire' exhibited a similar trend in proportional EL to 'Campbell 28' and 'Solar Set', but increased in proportional EL from 0.71 at 60°C to 1.02 at 62°C. All varieties surpassed the threshold for critical

damage (50% total EL) between 50 and 52°C with the exception of Nacgarlang, which had a proportional EL of 0.54 at the lowest tested RT of 50°C (Table 2.6).

Discussion

Root respiration rates across selected tomato varieties decreased to almost 0 at RT above 51°C, despite initially increasing when temperatures were increased from 48 to 51°C. The initial increase in respiration rate from 48 to 51°C may suggest that the varieties studied here had an optimal temperature of 51°C for root respiration. Previous studies of tomatoes grown at HRT over extended periods of time showed much lower RT optima for growth. For example, RT above 32°C over a 2 wk period limited total fresh and dry weight and leaf area of tomato (var. 'Vendor') seedlings (Hurewitz and Janes, 1983). Similarly, root and shoot growth were reduced when 'Amana Orange' and 'Solar Fire' tomato plants were grown at RT greater than 35°C for 8 h diurnally over 10 d (Guenthner and Erwin, *under review*). A slow reduction of growth potential is more likely in these scenarios, where carbohydrate allocation increasingly shifts to roots to balance elevated metabolic maintenance demands. For example, cucumber (*Cucumis sativus*) seedlings grown at 38°C RT increased their ^{14}C allocation to roots, but increased respiratory activity prevented its additional sequestration in root tissues (Du and Tachibana, 1994b). Therefore, tomatoes may have a short-term root respiration peak at 51°C and a long-term optima at 32°C.

The reduction in respiration rate between 51 and 57°C suggested that root systems surpassed the temperature threshold for respiration. Direct damage to root tissue occurring at HRT may be responsible this drop. Critical root damage at high

temperatures can occur within a matter of minutes rather than hours or days. For example, critical damage occurred to the roots of citrus between 51 and 54°C within 30 min (Ingram *et al.* 1986). LT_{50}s (the point of 'critical damage') of several woody plant species roots occurred between 45 and 58°C with a 20-35min (Ingram and Ruter, 2015). In our study, roots exposed to 54 and 57°C were 'soft' and discolored within 2hr, suggesting a loss of structural integrity may have occurred (Guenthner, *pers. obs.*). Similarly, discoloration occurred in 80% of hibiscus (*Hibiscus rosa-sinensis*) roots exposed to 50°C for 20 min (Lyles et al., 1992). The loss of root vigor at these temperatures parallels changes observed in the second experiment in this study testing root structural integrity through EL.

The increase in EL of tomato roots subjected to temperatures between 50 and 62°C for 30 min here is consistent with previous observations on woody and herbaceous plant species EL responses to HRT. Sigmoidal increases in electrolyte leakage were reported and modeled for a number of woody plant species (Ingram et al., 1986; Donovan et al, 1990; Sibley et al., 1998). The logistic trend in increasing EL observed in this study may capture the second half of a similar sigmoidal trend as the lowest temperature in out experiment was 50°C, as opposed to 20 or 25°C in other studies (Ingram *et al.* 1986; Sibley *et al.* 1999). Observed damage thresholds where the proportion of observed EL to maximum EL surpassed 0.50 was between 50 and 52°C following 30min, falling within the critical damage range previously reported for other plant species. Critical damage in other studies was reported as the temperature at which EL increased above its baseline threshold at non-stress temperatures by 50% (Ingram *et al.* 1986; Donovan *et al.* 1990; Sibley *et al.* 1999). For example, critical damage temperatures of roots of three

holly (*Ilex*) varieties were between 50.1 and 53.9°C when exposed for 30 min (Ruter 1993). Critical thresholds for Carrizo citrange (*Citrus sinensis x Poncirus trifoliata*) roots occurred at 51.6°C with a 20min exposure, while critical temperatures for the related Swingle citrumelo (*C. paradisi x P. trifoliata*) and sour orange (*C. aurantium*) were 53.5 and 52.5°C, respectively (Ingram *et al.* 1986). Reported critical temperatures of eight red maple varieties occurred between 52 and 53.5°C (Sibley et al. 1999). In order to further differentiate the critical damage thresholds of tomato varieties in our study, additional research employing a narrower series of temperature intervals and baseline EL under non-stress RT would be necessary. Further information would also lend itself to modeling of sigmoidal trends in EL as performed in Sibley et al. (1999) and Ingram et al. (1986).

Despite a lack of discernable differences in critical damage thresholds between tomato varieties in this study, RTs associated with maximum proportional damage to roots were differentiable. Tomato varieties such as 'Solar Fire' or 'Solar Set' did not reach their maximum proportional EL values until 62°C or higher, and may therefore have greater root membrane integrity than other varieties such as 'Nacgarlang' or 'Moskvich OG' that reached their maximum levels at 54°C. Additionally, the proportional EL of 'Nacgarlang' roots at 50°C was markedly higher at 0.54, compared to that of 'Solar Fire' roots at 0.27.

These trends do not reflect aboveground 'heat-tolerance' or 'sensitivity'. While 'Solar Set' and 'Solar Fire' are known for high fruit set capacity at high air temperatures (Barten *et al.* 1992; Scott *et al.* 2006), 'Nacgarlang' has also been characterized as a heat-tolerant variety based on photosynthetic and reproductive characteristics (Dane *et al.* 1991; Camejo *et al.* 2005). In our study, the higher proportional EL of 'Nacgarlang'

at lower temperatures suggested that previous tolerance classifications do not translate to root characteristics; at least for this variety. The temperature thresholds observed for maximum EL of the tomato varieties in this study are similar to thresholds reported for other plant species (Ingram *et al.* 1986; Donovan *et al.* 1990).

Both methods used in this study for assessing root responses to HRT had similar critical temperature thresholds for root respiration and EL. The 50% EL of all tomato varieties with the exception of 'Nacgarlang' (lower temperature) occurred between 50 and 52°C, while root respiration rates began to rapidly decrease above 51°C, suggesting a critical loss of cellular integrity and breakdown in metabolic activity occurred simultaneously above a 51-52°C root temperature. Variety-specific trends were only observed in EL data, suggesting this method of analysis may provide a greater degree of resolution than respiration rates for differentiating root tissue responses to high temperatures. However, EL measurements only indicated the degree of direct damage to roots, rather than the point at which metabolic activity is impaired. The proportional EL of varieties such as 'Solar Fire' and 'Solar Set' did not reach maximal values until RT of 62°C or greater; temperatures which are well beyond the observed 57°C threshold for the cessation of root respiration. Therefore, EL is best used as an indicator of physical integrity of roots, while the quantification of respiration is a better indicator of the temperature range at which they are active.

Conclusion

Here we demonstrated the negative impacts of HRT on the metabolic activity and physical integrity of tomato root systems. Root temperatures above 50°C, as observed in sun-exposed plastic containers, elicited rapid reductions in tomato root respiration and increased the severity of electrolyte leakage within 2 hr. These two responses suggested roots in containers can experience direct and indirect damage to roots. EL can provide high-resolution information on the critical temperature thresholds for root cellular integrity; while the measurement of root respiration rates can provide information on the temperatures at which metabolic activity remain active and/or impaired. Used in tandem, these two techniques can provide useful information on the temperature tolerance ranges of different species and varieties of herbaceous plants and contribute to the selection of heat-tolerant varieties based upon more than aboveground characteristics alone.

Table 2.1. Previous heat-response characterizations of tomato (*Solanum lycopersicum*) varieties used in this study based upon above-ground responses to high temperatures.

Tomato Variety	Heat Response	Characteristics Evaluated	Source
'Amana Orange'	Sensitive	Photosynthesis	Zhou et al., 2015
'Campbell 28'	Sensitive	Photosynthesis, Reproduction	Abdul-Baki, 1991; Camejo et al., 2005
'LA1994'	Tolerant	Photosynthesis	Zhou et al., 2015
'Moskvich OG'	Sensitive	Reproduction	Kamel et al., 2010
'Nacgarlang'	Tolerant	Photosynthesis, Reproduction	Camejo et al., 2005; Dane et al., 1991
'Saladette'	Tolerant	Reproduction	Abdul-Baki, 1991; Bita et al., 2011; Rudich et al., 1977
'Solar Set'	Tolerant	Reproduction	Abdul-Baki, 1991; Barten et al., 1992
'Solar Fire'	Tolerant	Reproduction	Scott et al., 2006

Table 2.2. Analysis of variance for the effects of temperature and tomato (*Solanum lycopersicum*) variety on whole root mass respiration rate normalized by fresh and dry weight ($\mu mol\ hr^{-1}\ g^{-1}$).

Variable	Factor	
	Fresh Weight	Dry Weight
Root Temperature (°C)	*** [z]	***
Tomato Variety	***	**
Root Temperature x Tomato Variety	n.s.	n.s.

[z] 'n.s.' indicates $p > 0.05$, '*' indicates $p \leq 0.05$, '**' indicates $p \leq 0.01$, '***' indicates $p \leq 0.001$.

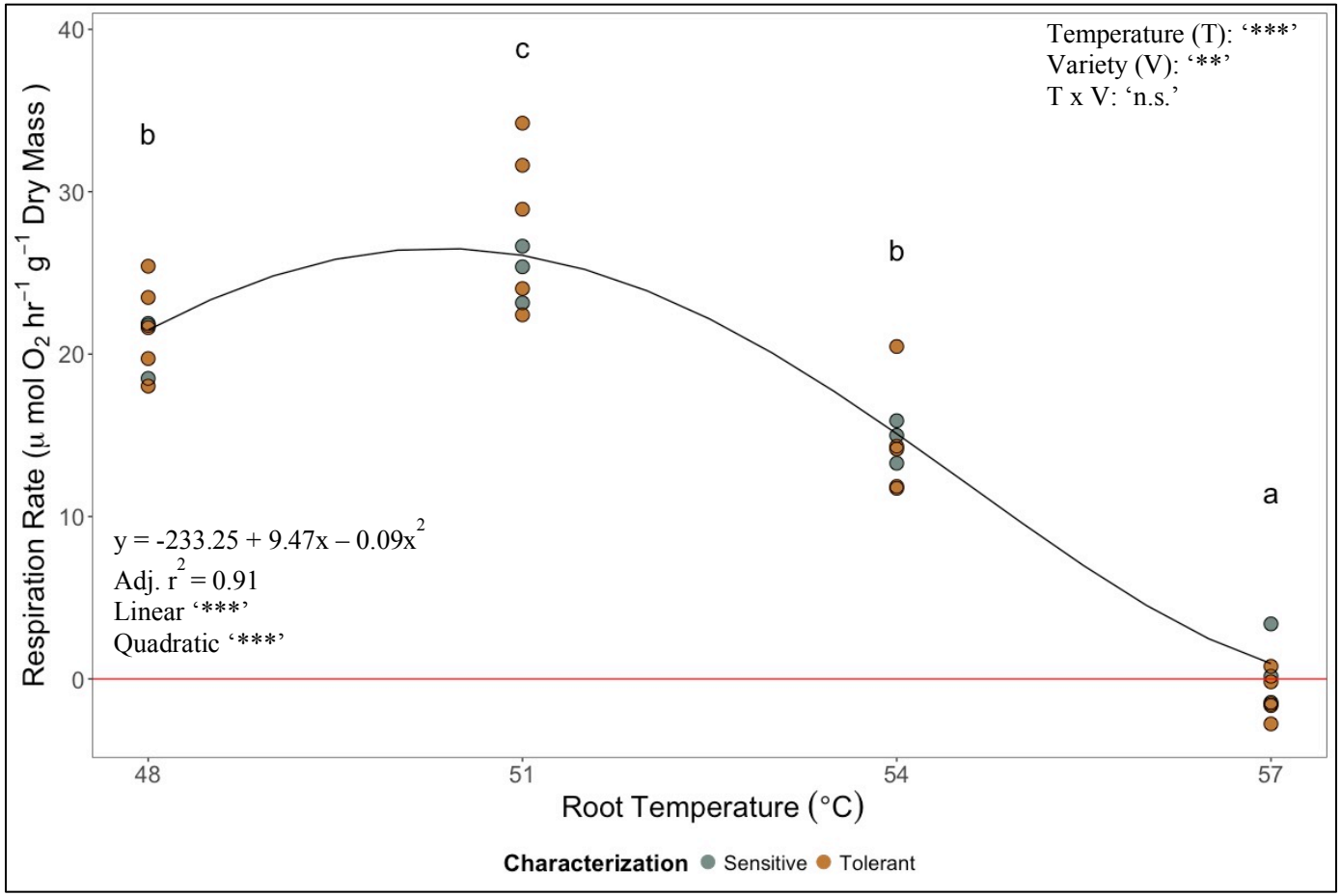


Figure 2.1. Effect of high root temperatures on respiration rate (μmol hr^{-1} g^{-1} dry weight) of eight tomato (*Solanum lycopersicum*) varieties varying in reported heat tolerance. Analysis was performed via one-way ANOVA with mean separation via Tukey's$_{HSD}$ ($p \geq 0.05$) and letters indicate differences between temperatures. Analysis of variance is presented with 'n.s.' = $p > 0.05$, '*' = $p \leq 0.05$, '**' = $p \leq 0.01$, '***' = $p \leq 0.001$.

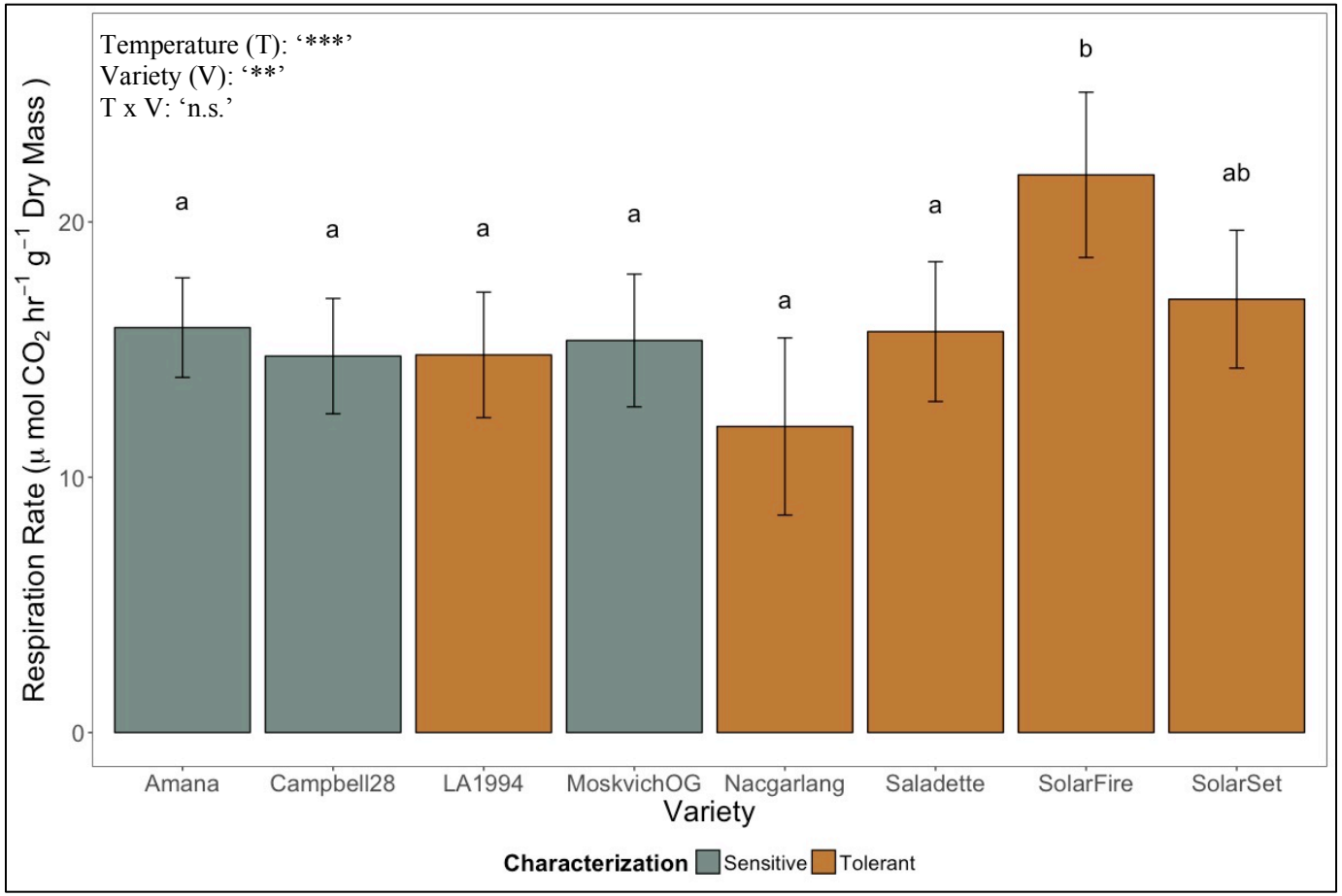


Figure 2.2. Whole root mass respiration rate (µmol hr^{-1} g^{-1} dry weight) of eight tomato (*Solanum lycopersicum*) varieties across four high root temperature treatments ranging from 48 to 57°C. Analysis was performed via one-way ANOVA with mean separation via Tukey's$_{HSD}$ ($p \geq 0.05$). Lowercase letters indicate differences between varieties and error bars represent +/- 1 SE. Analysis of variance is presented with 'n.s.' = $p > 0.05$, '*' = $p \leq 0.05$, '**' = $p \leq 0.01$, '***' = $p \leq 0.001$.

Table 2.3. Analysis of variance for the effects of root temperature and tomato (*Solanum lycopersicum*) variety on root mass electrolyte leakage normalized by fresh and dry weight (ms cm^{-3} g^{-1}).

Factor	Variable	
	Fresh Weight	Dry Weight
Root Temperature (°C)	*** [z]	***
Tomato Variety	***	***
Root Temperature x Tomato Variety	***	***

[z] 'n.s.' indicates $p > 0.05$, '*' indicates $p \leq 0.05$, '**' indicates $p \leq 0.01$, '***' indicates $p \leq 0.001$.

Table 2.4. Effects of temperature and variety on the electrolyte leakage of tomato (*Solanum lycopersicum*) root masses, normalized by dry weight (ms cm^{-3} g^{-1}). Mean separation within temperatures (down columns, capital letters) and within varieties (across rows, lowercase letters) was based upon two-way ANOVA with Tukey's$_{HSD}$ test for mean separation ($p \geq 0.05$). Bold lettering denotes 'heat tolerant' varieties.

Tomato Variety	Root Temperature						
	50	52	54	56	58	60	62
Amana Orange	2.38 Aa	2.86 Aa	4.34 Ab	4.85 ABb	6.98 Cc	4.86 Ab	5.33 ABb
Campbell 28	2.08 Aa	3.24 ABb	4.36 Acd	3.80 Abc	5.20 ABCd	4.89 Acd	4.79 Acd
LA1994	2.39 Aa	4.06 Bab	6.03 Bc	6.57 Cc	5.61 ABCc	5.67 Abc	6.77 BCbc
Moskvich OG	2.43 Aa	3.56 ABa	5.17 ABb	5.15 Bb	6.53 BCb	5.41 Ab	5.56 ABb
Nacgarlang	2.38 Aa	3.48 ABab	4.73 ABc	4.76 ABc	4.51 Abc	4.31 Abc	4.94 Ac
Saladette	2.11 Aa	2.79 Aa	4.78 ABbc	4.24 ABb	5.50 ABCbc	5.68 Ac	5.81 ABc
Solar Fire	2.01 Aa	3.25 ABab	4.44 Abc	4.98 Bc	5.21 ABCc	5.24 Ac	7.57 Cd
Solar Set	2.29 Aa	3.24 ABa	5.23 ABb	5.02 Bb	4.81 ABb	5.20 Ab	5.34 ABb

Table 2.5. Analysis of variance for the effects of temperature and variety on the proportion of maximum electrolyte leakage of tomato (*Solanum lycopersicum*) root masses, normalized by fresh and dry weight.

Variable	Factor	
	Fresh Weight	Dry Weight
Root Temperature (°C)	*** [z]	***
Tomato Variety	***	***
Root Temperature x Tomato Variety	***	***

[z] '***' indicates $p \leq 0.001$.

Table 2.6. Effects of temperature and tomato (*Solanum lycopersicum*) variety on the proportion of maximum electrolyte leakage root masses, normalized by fresh and dry weight. Mean separation within a temperature (down columns, capital letters) and within variety across temperature (across rows, lowercase letters) was based upon two-way ANOVA with Tukey's$_{HSD}$ test for mean separation ($p \geq 0.05$) following Arcsine transformation of proportional data. Bold lettering denotes 'heat tolerant' varieties based on previous research.

Tomto Variety	Root Temperature						
	50	52	54	56	58	60	62
Amana Orange	0.45 BCa	0.54 Aa	0.82 ABb	0.92 BCb	1.32 Cc	0.92 ABCDb	1.01 ABbc
Campbell 28	0.35 ABa	0.55 Aab	0.74 ABbcd	0.64 Abc	0.88 ABCd	0.82 ABcd	0.81 Acd
LA1994	0.36 ABa	0.68 ABab	0.90 BCbc	0.98 CDc	0.84 ABCbc	0.85 ABCbc	1.01 ABbc
Moskvich OG	0.45 BCa	0.65 Aa	0.95 BCb	0.94 BCb	1.20 Cb	0.99 BCDb	1.02 ABb
Nacgarlang	0.54 Ca	0.87 Bb	1.09 Cc	1.18 Dc	1.12 Cc	1.07 Dc	1.23 Bc
Saladette	0.40 ABa	0.52 Aa	0.90 Bbc	0.80 ABb	1.02 BCcd	1.21 CDd	1.22 Bd
Solar Fire	0.27 Aa	0.44 Aab	0.60 Abc	0.67 Abc	0.70 Ac	0.71 Ac	1.02 Bd
Solar Set	0.41 ABCa	0.58 Aab	0.93 BCc	0.90 BCc	0.86 ABbc	0.93 ABCDc	0.95 ABc

Chapter 3

Morphological and photosynthetic responses of tomato (*Solanum lycopersicum*) to high root zone temperatures

Containerized production systems are often above-ground and can be heated by air and sunlight, resulting in high root temperatures (HRT). We explored morphological and photosynthetic responses of tomato (*Solanum lycopersicum*) varieties previously characterized as heat-tolerant ('Solar Fire') or -sensitive ('Amana Orange') to diurnal, short-term HRT. Plants of each variety were grown for 8 h^{-1} d^{-1} with RT ranging from 25 to 60°C (5°C increments) for 10 d, and differences in plant morphology were noted. Plant height and leaf size decreased as temperature increased. Shoot and root fresh and dry mass gain decreased when RT increased from 35 to 50°C. Shoot and root fresh mass gain decreased from 15.85 g and 4.01 g at 35°C, respectively, to 2.29 g and 0.06 g, respectively, at 50°C. Shoot and root dry mass gain decreased from 1.67 g and 0.30 g at 35°C, respectively, to 0.41 g and 0.02 g at 50°C, respectively. Varieties did not differ in fresh and dry mass gain responses or percent reduction in shoot and root mass gain. In a second experiment, roots of both varieties were heated to 55°C for 260 min in the afternoon of one day and evaluated for changes in photosynthetic rate and stomatal conductance the following four days. Photosynthetic rate and stomatal conductance decreased after one 55°C RT exposure for 4 d compared to plants maintained at 25°C. 'Solar Fire' and 'Amana Orange' differed in percent reduction in stomatal conductance. Our findings suggested diurnal, short-term HRT negatively impacted growth and

photosynthesis regardless of reported above-ground heat tolerance, and that even one supraoptimal HRT event reduced photosynthetic activity for days.

Introduction

Plant production in containers can result in roots and media temperatures above what occurs in the ground. Plants have evolved a variety of strategies to avoid high root temperature (hereafter HRT) such as root growth under 'nursery' plants or deeper root growth to depths where temperatures are cooler (Jordan & Nobel 1984). Containerized production reduces a plants' ability to avoid HRT by elevating roots above the ground, often in dark-colored containers. Medium temperatures as high as 55-57°C were observed in above-ground black plastic containers in Minnesota and California (USA) in full sun in the afternoon (Guenthner, *pers. obs.*; Lyles et al., 1992). Daytime root temperatures peak during the afternoon, and those peaks can occur frequently depending on the weather (G. Guenthner, *pers. obs.*). 'Heat stress' has been defined as plant responses to temperatures 10-15°C above optimal temperatures (Wahid *et al.* 2007), however, other research suggests heat stress may occur in a narrower temperature window especially if stress events are repeated. For example, both root and shoot fresh weight decreased in lettuce (*Lactuca*) (25°C optimal growing temperature) when air temperature ranged from 28 to 36°C for 25 d (Lai & He 2016). Also, high temperatures can cause direct damage to roots within increasingly short periods of time as temperature event intensity increases. For example, predicted temperature thresholds for critical damage to the roots of Carrizo citrange (*Citrus sinensis x Poncirus trifoliata*) roots decreased from 158 ± 25 min at 45°C to 13 ± 5 min at 55°C (Ingram *et al.* 1986).

Previous research showed HRT is associated with reduced root and shoot growth. Creeping bentgrass (*Agrostis stolonifera* 'Penncross') grown with a 35°C RT (20°C air temperature) for 56 d had reduced root number and fresh weight relative to plants grown at 20°C RT (Xu & Huang 2000). Wheat (*Triticum spelta* 'USU Apogee') height and leaf size at 28-35°C RT was less than at 24°C RT (Monje *et al.* 2007). Roots and leaf dry mass, as well as root length and relative growth rate of hydroponically-grown olives (*Olea europea* 'Arbequina') were reduced when grown with a 37°C RT compared to plants grown with a 25°C RT (Benlloch-González *et al.* 2017). Cucumber (*Cucumis sativus*) root, stem, and leaf dry weight was reduced when roots were exposed to 38°C for 8-16 d, relative to plants grown with a 25°C RT (Du & Tachibana 1994a).

Differences in fresh and dry mass are related to effects of HRT on photosynthetic rate (P_n) and stomatal conductance (g_s). Bentgrass ('Penncross') leaf P_n and g_s were lower when RT was 35°C RT than at 20°C RT, when air temperature was maintained at 20°C (Xu & Huang 2000). Reduced P_n and g_s were associated with reductions in leaf relative water content of butterhead lettuce (*Lactuca sativa*) grown with fluctuating diurnal RT (23 and 40°C; He et al., 2001). Cucumber (*C. sativus*) whole plant, root, and leaf fresh weight, and P_n and g_s were reduced in plants grown at 38°C versus 30°C RT for 10d (Nada *et al.* 2003). Reduced rice (*Oryza sativa*) P_n and g_s occurred after exposure of roots to 37°C RT for 21 d (Arai-Sanoh *et al.* 2010b).

The mechanisms by which HRT reduces growth and photosynthesis are not well understood. Reactive oxygen species (ROS) accumulation resulting from HRT can damage cell membranes and impact metabolic activity in shoots. For example, increased malondialdehyde (MDA; a product of lipid peroxidation by ROS) content in

leaves of bentgrass (*A. stolonifera* var. 'Penncross') accompanied reductions in photochemical efficiency over a 40 d period of growth at with 35°C RT (Liu & Huang 2004).

The stomatal closure and subsequent limitation of P_n may contribute to reduced mass (as noted above), and may be associated with changes in plant water status or stress-triggered abscisic acid (ABA) signaling. Olive (*O. europea* 'Arbequina') seedlings grown in a 37°C hydroponic solution for 33 d had limited uptake and transport of potassium (K^+; important to root tissue osmotic exchange), that may contribute to reduced root and shoot dry matter gain (Benlloch-González *et al.* 2017). ABA is associated with stomatal closure and limitation of P_n (Downton *et al.* 1988; Popova *et al.* 1996). Reductions in g_s and P_n of six Cucurbit (*Cucumis*) species grown at RT above or below optimal temperatures were associated with higher levels of ABA) in leaf tissues (Zhang *et al.* 2008). Similarly, increased leaf ABA concentrations accompanied reductions in g_s and P_n of cucumber (*C. sativus* 'Suyo') leaves following exposure to RT of 38°C for 10 d relative to plants grown at a 30°C RT (Nada *et al.* 2003). Whether direct damage to roots occurring during a short-term HRT exposure contributes to ABA biosynthesis in roots, and subsequent transport to leaves where g_s and P_n are limited, has not been explored.

Variation in HRT responses among related species or varieties that differ in heat tolerance is poorly documented. The root relative growth rate (mg g^{-1} d^{-1}) and length of the bentgrass species *Agrostis scabra* (indigenous to geothermal soils) remained higher than those of the related 'cool-season' species *A. stolonifera* (var. 'L-93' and 'Penncross') at 37°C RT over 28 d (Rachmilevitch, Lambers, *et al.* 2006). Kentucky Bluestem (*Poa pratensis*) varieties differed in root and shoot electrolyte leakage

when grown with 35/30°C air temperatures over 7-28 d (Zhang & Du 2016). However, the critical thresholds for root electrolyte leakage from roots of nine maple (*Acer*) varieties (woody plant species) did not differ substantially (Sibley *et al.* 1999).

The objectives of this research were 1) to determine growth responses of tomato varieties that differ in above-ground heat tolerance to prolonged exposure to diurnal HRT, and 2) to explore the effect of a single short-term, afternoon HRT event on P_n and g_s activity during and following the stress. We anticipated that increasing diurnal RT would negatively impact tomato growth and that exposure to acute HRT would reduce photosynthetic activity, regardless of variety.

Materials and Methods

Experiment I: Morphological Responses to Diurnal HRT

Two tomato varieties previously characterized as heat-tolerant ('Solar Fire') or heat-sensitive ('Amana Orange') were selected. 'Solar Fire' was identified as 'heat-tolerant' based on fruit-setting ability at air temperatures above 32°C (Scott *et al.* 2006). 'Amana Orange' was identified as 'heat-sensitive' based on maximum quantum efficiency of photosystem II (F_v/F_m) at high air temperatures (Zhou, Yu, *et al.* 2015). Additionally, these two varieties differed in root electrolyte leakage responses to short-term HRT as determined in a previous experiment (Guenthner and Erwin, *see Chapter 2*). Seed of 'Solar Fire' and 'Amana Orange' were obtained from the University of Florida (Gulf Coast Research and Education Center; Balm, FL) and from Tomato Grower's Supply (Fort Myers, FL). Seeds were sown into 50 cell trays (one seed per cell; vol. 75 mL; TO Plastics, Clearwater MN) in a soilless media (SunGro SS#8-F2; Agawam, MA)

and were lightly covered with medium grade vermiculite (3 mm). Initial sown seeds were placed in a mist greenhouse maintained at 24 ± 1.2°C day/night temperatures under natural daylight conditions with 100 ± 20 μmol m^{-2} s^{-1} supplemental light (when light levels were below 420 μmol m^{-2} s^{-1}) for 9 d. Trays were initially watered manually and were watered thereafter with periodic automated mist for 9 d (20-30 sec every 15 min). Following germination (emergence of the radicle), seedlings were transferred to a greenhouse under natural daylight conditions with supplemental HID light (100 ± 10 μmol m^{-2} s^{-1}) extended to an 18hr day (0800-0200HR; 13.4 ± 3.0 mol m^{-2} d^{-1}), and constant temperatures of 25.2ºC ± 1.0°C. Additional seeding and germination was performed under these greenhouse conditions without the use of the aforementioned mist greenhouse. Plants were watered as needed to maintain a moist media conditions and fertilized through the irrigation water with Peters Excel CalMag 15-5-15 (ICL Specialty Fertilizers; Summerville, SC) at a concentration of 250 ppm N.

Plants with two true unfolded leaves (petiole angle ≥ 45°) were transferred over a 11 d period with 1-2 d between each replicate group to an environmental growth chamber (Environmental Growth Chambers; Chagrin Falls, OH) with a 12-hr photoperiod (0500-1700HR) with an irradiance of 400 ± 30 μmol m^{-2} s^{-1} provided with fluorescent (75% wattage) and incandescent lamps (25% total wattage) 1 d prior to the initiation of RT treatments. Chamber relative humidity was 50% and day/night temperature regime was 25/20°C, respectively.

After one day in this chamber, plants of each variety were transferred to individual RT treatment water baths in a second growth chamber (same manufacturer). This chamber was set to constant 24°C with a 12 hr photoperiod (400 ± 20 μmol m^{-2} s^{-1})

provided with fluorescent lamps and a relative humidity of 50%. Undisturbed plugs containing whole plants and roots were inserted into a single cell of a waterproof 50-cell tray that was approximately 80% submerged (from the bottom) in water baths heated to different target treatment temperatures (25, 30, 35, 40, 45, 50, 55, 60°C). Plastic storage bin water baths (vol. 27 L each; Rubbermaid, Atlanta GA) were heated and maintained at desired temperatures using a sous-vide immersion heater and circulator (Gourmia GSV140; Brooklyn, NY). A digital temperature probe (Quartz Digi-Thermo, Traceable Products; Webster TX) was inserted 5 cm deep on the edge of sample root masses in each treatment to verify RTs. Root mass temperatures for each treatment group were 25.8 ± 0.2°C, 30.3 ± 0.2°C, 35.0 ± 0.2°C, 40.0 ± 0.2°C, 45.4 ± 0.2°C, 50.1 ± 0.2°C, 55.7 ± 0.3°C, 59.6 ± 0.4°C, respectively. Plants were maintained in treatment baths 8 h^{-1} d^{-1} (0900-1700HR) for 10 d to simulate mid-day heating. At the end of each daily treatment period, plants were returned to the first growth chamber for a 12 h dark period at maintained at 20°C. Plants were watered as needed to maintain moist media conditions using Peters Excel CalMag 15-5-15 (250 ppm N; ICL Specialty Fertilizers; Summerville, SC).

On day 11, plants were destructively sampled. Stem height, unfolded leaf number, third unfolded true leaf length and width, and shoot and root fresh mass were recorded. Presence or absence of leaf edge browning was also noted. Roots were washed free of media and patted dry with paper towels prior to determining fresh mass. Shoot and root samples were dried in an oven (Hotpack Corp., Philadelphia PA) at 65°C for 3+ days before measuring dry mass. Additional plants were grown with treatment plants and destructively sampled for stem height and root and shoot fresh and dry mass at the

initiation of the experiment (2 unfolded leaf stage) to determine the change in fresh and dry mass gain of treatment plants following treatments.

This experiment was designed as a completely randomized statistical design in a factorial arrangement; temperature and varieties were main effects. Values obtained for plant height, leaf count and fresh and dry shoot and root mass were analyzed via using ANOVA. The presence/absence of leaf browning was analyzed using a Pearson Chi-Square test. Shoot and root growth was evaluated in two different ways during data analysis. In the first, change in mass was determined by subtracting the initial mean shoot and root mass from plant masses after the 10 d RT treatments. In the second, the percent reduction in mass, determined by dividing shoot and root masses of plants grown at 30°C and higher RT by the mean shoot and root masses of plants grown at a 25°C RT (unstressed). These percent data were arcsine-transformed prior to ANOVA. All data were analyzed using SPSS Statistics v. 24 (IBM Co.; Armonk NY). Outliers were identified using the SPSS 'Explore' function and removed where values lay outside the interquartile range by three times its inner range. Tukey's$_{HSD}$ was used for mean separation. To determine regression lines of best fit, shoot and root mass gain data was square root transformed and converted back for visual representation.

Experiment II: Photosynthetic Responses to Acute HRT

The two tomato varieties selected above were sown and germinated as summarized in Expt. 1. Following germination, initial growth was in a greenhouse under natural daylight conditions with 100 µmol m^{-2} s^{-1} (± 15 µmol m^{-2} s^{-1}) supplemental high intensity discharge lighting extended to 18 h^{-1} d^{-1} (0800-0200 HR; 13.4 ± 3.0 mol m^{-2} d^{-}

^{1}), and constant 28.8ºC ± 1.2°C. Plants were watered as needed to maintain moist media and fertilized through the irrigation water with Peters Excel CalMag 15-5-15 (250 ppm N; ICL Specialty Fertilizers; Summerville, SC).

Plants were transferred to 10.54 cm diameter plastic pots at a four-leaf stage of development (3 wk after germination) and maintained under the same greenhouse conditions for an additional 2 wk. Three days prior to RT treatments, plants were moved to a growth chamber (Environmental Growth Chambers; Chagrin Falls, OH) with a 28°C air temperature, 50% relative humidity setpoint, and an 18 hr photoperiod (330 ± 10 umol m^{-2} s^{-1}). Plants were set in trays containing 1 cm water supplemented with Peters Excel CalMag 15-5-15 (250 ppm N; ICL Specialty Fertilizers; Summerville, SC) to maintain consistent, moist media.

Six plants of each variety were randomly distributed into pre-cut bin lids covering two water baths (vol. 27 L each; Rubbermaid, Atlanta GA) heated to 55°C using immersion heaters and circulators (Gourmia GSV140; Brooklyn, NY). Pots were initially wrapped with two plastic bags and inserted into a second 10.54 cm diameter plastic pot to ensure roots and media were not flooded with water when suspended in water baths; the media surface was exposed to the open air. A HRT of 55°C was chosen to represent a critical, supraoptimal RT based on temperature thresholds for tomato root respiration and electrolyte leakage observed in a previous experiment (Guenthner and Erwin, *see Chapter 2*). Temperatures of 55.5°C ± 0.5°C on the outer edge of root mass were confirmed by inserting a digital temperature probe (Quartz Digi-Thermo, Traceable Products; Webster TX) 5 cm deep. RT treatments were applied from 1200-1620HR to represent an afternoon heating event. Following the RT treatment period, plants were

removed from water baths and maintained under ambient conditions in the growth chamber (see above). Six plants of each variety did not receive a HRT (at RT of 25.0°C ± 0.8°C) to serve as a control of un-treated plants for comparison and were kept in the same growth chamber as HRT-treated plants.

P_n and g_s were measured between 1345 and 1630HR on the 4^{th}-5^{th} leaflet on the second unfolded leaf from the apical meristem using a LI-6400XT Photosynthesis Machine (LiCor Biosciences; Lincoln, NE), with sampling order randomized across treatments and varieties. RT treatments plants were sampled while in treatment baths on the first sample day. The leaf measurement cuvette of the LI-6400XT was programmed to maintain 300 μmol m^{-2} s^{-1} irradiance, 400 ppm CO_2 and an air flow rate of 500 μmol s^{-1}. Relative humidity was not regulated and was 58 ± 3%. Measurements were taken after a 5-6 min period when P_n had stabilized.

Additional P_n and g_s measurements were made daily at the same time for 4 d following the initial RT treatment as well as on untreated plants. Measurements on each plant on the same leaf and leaflet as the first day and sampling order was re-randomized daily. Both heat-treated and un-treated plants were grown in the growth chamber under the previously specified environmental conditions and RT was 25.9°C ± 0.5°C across treatment groups after the initial RT treatment.

The experiment was designed with a split-plot, repeated measures statistical design with RT, variety, and day of measurement as the main effects. Data were analyzed using analysis of variance. Daily P_n and g_s values from high RT treatment plants were divided by corresponding daily means of untreated plants to calculate the daily percent reduction in P_n and g_s. These proportions were normalized using an arcsine

transformation prior to analysis of variance. All data was processed and analyzed using SPSS Statistics v. 24 (IBM Co.; Armonk NY). Outliers were identified using the SPSS 'Explore' function and were removed when identified (data which lay outside the interquartile range by three times its inner range). Tukey's$_{HSD}$ was employed for mean separation.

Results

Experiment I: Morphological Responses to Diurnal HRT

Tomato plants visibly changed when exposed to RT of 40°C and higher (Fig. 3.1 and Fig. 3.2). Differences between varieties' appearances in response to increasing RT were not apparent. Shoots of plants grown with a 25-35°C RT were taller and had greater leaf length and width than those grown at 45°C and higher RT, giving the appearance of fuller growth; visible root distribution and density appeared highest within the 25-35°C RT range (Fig. 3.1A). Plants grown at 45-60°C RT showed signs of stunted growth and chlorosis/leaf browning by the end of the treatment period (Fig. 3.1B). Lower leaf edge browning was present on two plants with a 45°C RT and present on all plants of both varieties with a 50°C and higher RT. Pearson Chi-Square analysis indicated RT affected leaf browning ($p < 0.001$), but varieties did not differ. Plants grown at 55-60°C had a loss of stem integrity (fell over) 4-6 days after RT treatments were initiated (G. Guenthner, *pers. obs.*). Root distribution was confined to the upper ½ of the growing media with a 40-45°C RT, while few roots were visible with a 50-60°C RT (Fig. 3.2).

Temperature and variety interacted ($p \leq 0.01$) to affect unfolded leaf number (Table 3.1A). For instance, leaf number was higher for 'Amana Orange' between RT of 25 and 45°C than at RT of 50-60°C (Table 3.3). In contrast, leaf number of 'Solar Fire' plants did not differ between 25 and 40°C, above which leaf number decreased (Table 3.3). 'Solar Fire' leaf number was lower than 'Amana Orange' at RT of 25 to 45°C; however at 50°C RT and above, varieties did not differ (Table 3.3). While unfolded leaf number varied by less than three leaves across RT treatments, differences in leaf number were notable between low and high RT treatments (Fig. 3.1).

Temperature and variety affected leaf length ($p \leq 0.001$) and width ($p \leq 0.001$) independently (Table 3.1A). Leaf length and width were greatest when RT was 25-40°C and lowest when RT was 50-60°C across varieties (Table 3.2). Across RT, 'Solar Fire' plants had greater leaf length and width than 'Amana Orange'. Stem height differed between RT ($p \leq 0.001$), but not among varieties (Table 3.1A). Stem height was similar when RTs were 25-40°C, but decreased when RT was 45°C and higher (Table 3.2).

Shoot and root fresh mass gain were impacted by RT ($p \leq 0.001$), but not variety (Table 3.1A). Shoot fresh mass gain decreased with RT above 35°C; for instance, it was 15.85g at 35°C RT and 0.92g at 60°C RT (Fig. 3.3). Shoot fresh mass gain did not differ among plants grown with a 25-35°C RT or among plants grown with RT of 50-60°C (Fig. 3.3A). Shoot dry mass gain decreased from 1.67g at 35°C RT to 0.30g at 60°C RT (Fig. 3.3B). Root fresh mass gain was highest with a 25-35°C RT, and decreased as RT increased (Fig. 3.3C). At RT of 55 and 60°C, root fresh mass gain was less than 0 (Fig. 3.3C). Root dry mass gain decreased from 0.30g to 0.02g as RT increased from 35 to

50°C, but it did not differ statistically at RT between 25 and 35°C or between RT of 50 to 60°C (Fig. 3.3D).

The percent reduction in shoot fresh mass of tomatoes grown with RT above 25°C was impacted by RT ($p \leq 0.001$), but not variety (Table 3.1A). At RTs of 30 and 35°C, shoot fresh mass was greater than at 25°C RT by 2.8 to 3.6% respectively (Fig. 3.4A) However it was 24.5% lower at 40°C and up to 86% lower at RT of 60°C than that of plants grown at 25°C (Fig. 3.4A).

Percent reduction in shoot dry mass was affected by RT ($p \leq 0.001$) but not by variety (Table 3.1A). Shoot dry mass of plants with 30 and 35°C RTs differed from plants grown with a RT of 25°C by less than 1%, but shoot dry mass decreased by 75% when RT was 60°C (Fig. 3.4B). Reductions in root fresh and dry mass were also influenced by RT ($p \leq 0.001$ in both cases), but not variety (Table 3.1A). Root fresh mass at 30 and 35°C differed by less than 3% from root fresh mass at 25°C, but was by 35% lower at 40°C and 97% lower with RT of 60°C (Fig. 3.4C). Root dry mass at 30 and 35°C was greater than 25°C by 1.18 and 4.06%, respectively (Fig. 3.4D). However root dry mass decreased up to 93% with RT of 60°C compared to plants grown at 25°C (Fig. 3.4D).

Experiment II: Photosynthetic Responses to Acute HRT

Exposure of roots to 55°C for 4.33 hr on one afternoon reduced P_n and g_s of 'Amana Orange' and 'Solar Fire' for 4 d. P_n and g_s were impacted by an interaction between RT and measurement day ($p \leq 0.05$ and $p \leq 0.001$, respectively) (Table 3.1B). P_n

of untreated plants (26°C RT) was lower on the last day of measurement at 10.32 µmol CO_2 m^{-2} s^{-1} than the first day at 12.52 µmol CO_2 m^{-2} s^{-1}, but did not differ from values on days in-between (Fig. 3.5A). P_n of plants exposed to a 55°C RT on the first day was 11.32 µmol CO_2 m^{-2} s^{-1}, but dropped to 7.59 µmol CO_2 m^{-2} s^{-1} on the second day and did not increase the remaining three days (Fig. 3.5A). G_s of untreated plants was higher the first two days of measurement than the following three days, but the g_s of 55°C RT-treated plants followed the same trend observed for P_n (Fig. 3.5B); g_s of high RT treatment plants was highest on the first day at 0.28 mol H_2O m^{-2} s^{-1}, and decreased to 0.08 mol H_2O m^{-2} s^{-1} on the second and did not increase thereafter.

The percent reduction in P_n between plants maintained at 25°C RT and those with 55°C RT differed across measurement day ($p \leq 0.001$) but not variety (Table 3.1B). On the first day of measurement, P_n was 10% lower on 55°C RT exposed plants than untreated plants, but was reduced by 37% the second day. The percent reduction in P_n on subsequent days of measurement did not differ statistically from day 2, though values were lower (Fig. 3.6A).

Variety and measurement day interacted ($p \leq 0.01$) to affect the percent reduction in g_s (Table 3.1B). A 61% reduction in g_s was observed on 'Amana Orange', reduction in g_s compared to unstressed plants increased to 87% on the second day of measurement and did not increase thereafter (Fig. 3.6B). The reduction in 'Solar Fire' g_s after plant roots were exposed to 55°C was initially lower than 'Amana Orange'; 'Solar Fire' g_s on the first day was reduced by 27%, but climbed to 76% by the second day (Fig. 3.5B). Unlike 'Amana Orange', the percent reduction in g_s of 'Solar Fire' did not change after the

second day of measurement. However, the percent reduction in g_s between the two varieties only differed statistically on the first day of measurement (and treatment).

Discussion

The trends observed in both variety fresh and dry mass gain at high RTs in Experiment 1 reinforced observations that high RT can limit growth. Tomatoes grown with RT between 25 and 35°C had the greatest shoot and root mass, suggesting the optimum RT for growth of these varieties was in this range. Hurewitz and Janes (1983) reported the tomato 'Vendor' had an optimum RT of 32.2°C based on a constant, 14 day exposure; a duration that was longer than in this paper. Klock et al., (1977) had similar results and noted the shoot dry mass of 'Moneymaker' tomato plants grown at constant 35°C RT for 19d was lower compared to plants grown at 25°C. In containerized production, plants experience diurnal fluctuations in RT (Nambuthiri *et al.* 2015). While roots may experience temperatures above growing optima for short periods during the day, a return to cooler night temperatures may allow recovery and acclimation for subsequent high RT events. For example, a 20°C increase in diurnal air temperatures increased the critical damage threshold of root cortical cells of the cacti *Nopalea cochenillifera* and *Opuntia robusta* by 3.4°C (Nobel & Zutta 2008). A similar acclimatory response of roots to repeated temperature conditioning in this study might explain why growth was not hampered by 8h d^{-1} at RT as high as 35°C.

RTs above a certain temperature threshold can have long-term negative impacts on root and shoot growth regardless of diurnal fluctuation. Critical temperature thresholds (50% loss of cellular solutes) between 51 and 54°C RTs with a 30min exposure

were reported for three citrus varieties (*Citrus* and *Citrus x Poncirus*) (Ingram *et al.* 1986). Likewise, critical damage thresholds for nine maple (*Acer*) varieties were reported between 52 ± 0.8°C and 53.3 ± 0.5°C (Sibley *et al.* 1999). We previously determined critical damage thresholds for excised roots of 'Solar Fire' and Amana Orange' were between 50 and 52°C with 30min of exposure (Guenthner and Erwin, *see Chapter 2*). This suggested the RTs above 55°C may have damaged roots the first day of the RT treatment, and may explain why shoot and root mass gain were limited with increasing RT.

In this study, RT above 35°C reduced shoot and root fresh and dry mass gain, with little mass being added above 50°C RT. The lack of growth may be due in part to reduced P_n and increased respiration resulting in carbohydrate stress in heat-stressed plants. For example, ^{14}C sequestration of cucumber plants (*C. sativus*) was reduced in leaf and root tissues as elevated respiration rates offset increased belowground ^{14}C allocation after a 9d 38°C RT (Du & Tachibana 1994b). Similarly, carbon allocation to shoots in creeping bentgrass (*A. scabra and A. stolonifera* var. 'Penncross') was reduced by 37°C RT for 28d (Rachmilevitch *et al.* 2015). Root fresh and dry mass loss with RT of 55-60°C in this study may therefore reflect both respiratory exhaustion of resources in roots and/or the loss of solutes and death of tissue caused by acute high RT damage. Here, roots appeared to cluster near the media surface when RT was 40-45°C. Hurewitz and Janes (1983) observed a similar clustering of roots near the media surface when tomato 'Vendor' were grown with at RT above 32°C.

Variation in growth between 'Amana Orange' and 'Solar Fire' only differed with in leaf length and width data in the first experiment. The lack of discernable differences in growth responses to high RT between varieties suggested previously observed characteristics of 'heat-tolerance' or 'sensitivity' of above media responses did not associate with high RT responses in this study (Scott *et al.* 2006; Zhou, Yu, *et al.* 2015). Both of the aboveground characteristics previously used to characterize these two varieties, fruit set and F_v/F_m at high air temperatures, likely had little role to play in how the roots responded to high RT. High RT was not associated with a reduction in F_v/F_m in previous research, even when P_n and g_s were reduced (Nada *et al.* 2003).

A single, short duration exposure to high RT can limit P_n in tomato plants for at least four days. The reduction in P_n between the first and subsequent days of measurement in plants exposed to 55°C RT suggested a delayed but sustained response to the high RT. The reduction in P_n echoes previous observations of plant photosynthetic responses to high RT exposure, however those reductions were made following extended exposure periods of days to weeks at lower RT than the RT used in this study (Dodd *et al.* 2000; Xu & Huang 2000; He *et al.* 2001; Nada *et al.* 2003). For example, canopy P_n of bentgrass (*A. stolonifera* 'Penncross') decreased between 20 and 50 d of growth at RT of 35°C (Xu & Huang 2000). The aforementioned studies of high RT impacts on photosynthesis have not examined the effects of periodic diurnal acute high RT exposure, and how post-exposure periods of response and recovery contribute to long-term changes in growth. Given our previous observations that RT in containers in MN and CA can reach temperatures >50°C, we believe that the impact of periodic high RTs on P_n may be greater than appreciated.

Long-term reduction of g_s to a single 55°C RT exposure suggested these responses may be linked – as reduced g_s can limit leaf gas exchange and P_n. The basis for the reduction in g_s and P_n at 55°C RT-treated may be associated with an increase in abscisic acid (ABA) synthesis, transport, and/or increase in free ABA present in leaf cells triggered by a stress event (Wilkinson & Davies 2002). Elevated concentrations of ABA are associated with exposure of plants to RT outside their optimal growing range (Nada *et al.* 2003; Arai-Sanoh *et al.* 2010a). For instance, leaf ABA content of cucumbers (*C. sativus*) grown at RT of 38°C increased first 8d of exposure, and remained elevated through the remainder of the study (Nada *et al.* 2003).

Observed reductions in P_n and g_s of un-stressed plants may be attributed to either leaf aging or thigmotropic effects from handling the same leaf repeatedly. Leaf P_n decreases as a plant ages after a period of time. For example, P_n of the third unfolded leaf on wheat plants peaked at 7d and decreased (Suzuki *et al.* 2008). However, the lack of change in absolute P_n and g_s of leaves on high RT-treated plant may indicate that either an alternative factor impacted leaves of control plants over time, or that the P_n and g_s depression caused by high RT exposure produced a response that exceeded age-associated declines in photosynthetic activity.

While ‘Amana Orange’ and ‘Solar Fire’ plants did not differ in P_n, g_s, or daily percent reduction in P_n, varietal differences occurred in the daily percent reduction in g_s. On the first day of measurement, also the day of high RT treatment, g_s of high RT ‘Amana Orange’ was 61% lower than un-stressed plants, compared to only a 27% difference between stressed and non-stressed ‘Solar Fire’ plants. The higher reduction in

'Amana Orange' g_s on the first day may suggest a more rapid response to high RT than 'Solar Fire' that may be associated with different responses to stress-induced ABA synthesis and activity. While differences in g_s were not statistically significant after the first day, it is worth noting that the percent reduction in g_s of 'Amana Orange' plants was higher than 'Solar Fire' plants for the first four days. This suggests that there may be a difference in the sensitivity of this variety that was obscured by the relatively low number of replicates in this study.

Conclusion

The production of horticultural crops in containerized systems can routinely expose plants to supraoptimal root temperatures during the summer growing season. While past research has suggested high RT negatively impacted plant growth, further exploration of the temperature thresholds and modes of physiological response amongst different varieties of herbaceous plants remains scarce. The results of this study demonstrate that high RT exposure can negatively impact both aboveground and belowground growth of young tomato plants. Established optimal temperature thresholds remain low, even with high temperature exposure only occurring for a portion of each day. Previous characterizations of heat tolerance amongst horticultural crops based upon aboveground attributes may not be enough to differentiate tolerance in the more sensitive root systems, leaving a gap in our methods for selecting plants that can tolerate more extreme growing conditions. Additionally, our research suggested that even one high RT event can reduce the photosynthetic potential of affected plants for days. The degree to which such commonplace heat events limit growth of horticultural crops is not well understood. In

addition, greater attention should be given to ameliorating root heat stress in production systems if growers wish to maximize their production potential. Additional research on how these cyclic or single exposure events can reduce plant growth and/or productivity is merited.

Figure 3.1. Representative images of shoot and growth of the tomato (*Solanum lycopersicum*) variety 'Solar Fire' following diurnal exposure to root temperatures between 25 and 60°C for 8 h d^{-1} over a 10 d treatment period. Tomato varieties ('Solar Fire' and 'Amana Orange') did not differ in appearance within temperatures. Pictures A and B are not to matching scale, but the same 40°C RT plant was used in both pictures provide a visual reference across images.

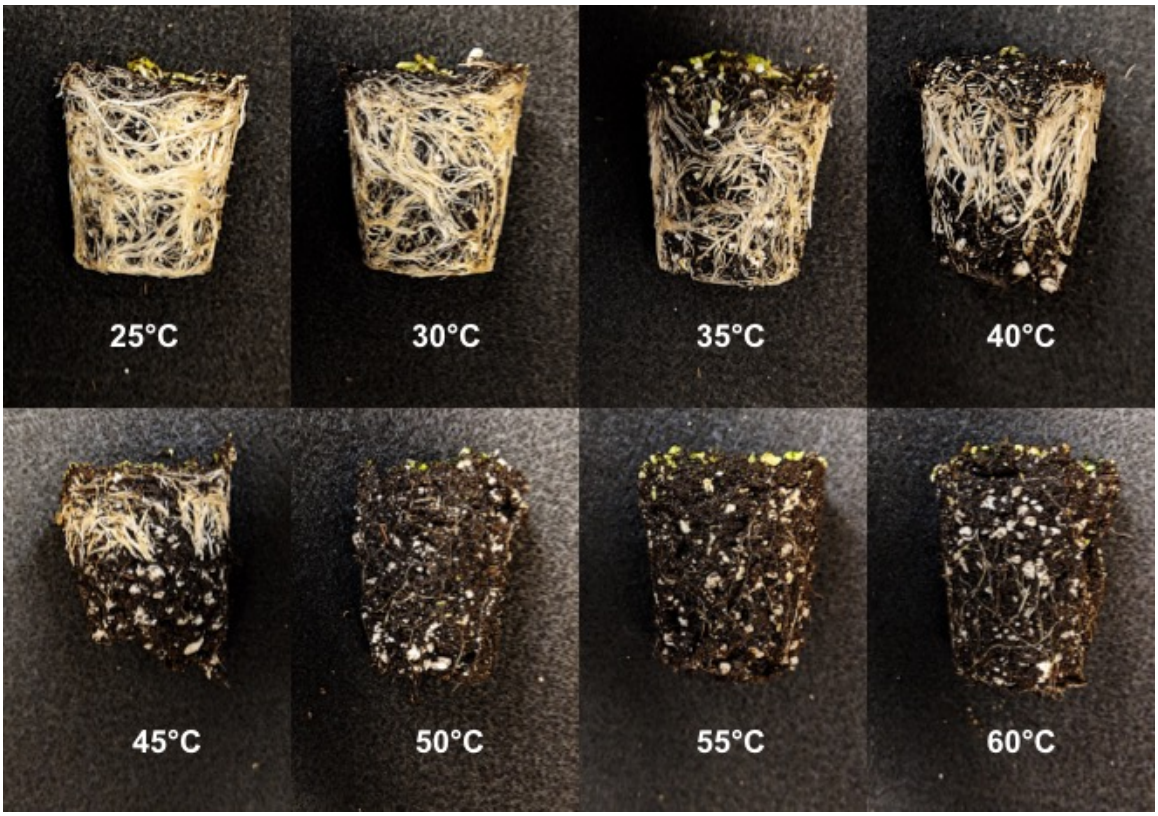


Figure 3.2. Representative images of root growth of the tomato (*Solanum lycopersicum*) variety 'Solar Fire' following diurnal exposure to RT between 25 and 60°C for 8 h d^{-1} over a 10 d treatment period. Tomato varieties ('Solar Fire' and 'Amana Orange') did not differ in appearance within temperatures.

Table 3.1. Analysis of variance for the impacts of tomato (*Solanum lycopersicum*) variety and root temperature on characteristics of growth (A) and photosynthetic activity (B) impacted by tomato variety and root temperature. Asterisks indicate level of significance following analysis via one-way ANOVA with Tukey's HSD for mean separation ('n.s.' indicates $p > 0.05$, '*' indicates $p \leq 0.05$, '**' indicates $p \leq 0.01$, '***' indicates $p \leq 0.001$).

A. Experiment 1

Variable	Factor		
	Root Temperature (°C)	Variety	Root Temperature x Variety
Unfolded Leaves	***	***	**
Stem Height (cm)	***	n.s.	n.s.
Third Leaf Length (cm)	***	***	n.s.
Third Leaf Width (cm)	***	***	n.s.
Shoot Fresh Mass Gain (g)	***	n.s.	n.s.
Shoot Dry Mass Gain (g)	***	n.s.	n.s.
Root Fresh Mass Gain (g)	***	n.s.	n.s.
Root Dry Mass Gain (g)	***	n.s.	n.s.
Percent Reduction in Shoot Fresh Mass	***	n.s.	n.s.
Percent Reduction in Shoot Dry Mass	***	n.s.	n.s.
Percent Reduction in Root Fresh Mass	***	n.s.	n.s.
Percent Reduction in Root Dry Mass	***	n.s.	n.s.

B. Experiment 2

Factor	Variable			
	Photosynthetic Rate (P_n) (umol CO_2 m^{-2} s^{-1})	Stomatal Conductance (g_s) (mol H_2O m^{-2} s^{-1})	% Reduction in P_n	% Reduction in g_s
Root Temperature (RT)	***	***	---	---
Tomato Variety (TV)	n.s.	n.s.	n.s.	**
Measurement Day (MD)	***	***	***	***
RT x TV	n.s.	*	---	---
RT x MD	*	***	---	---
TV x MD	n.s.	n.s.	n.s.	**
RT x TV x MD	n.s.	n.s.	---	---

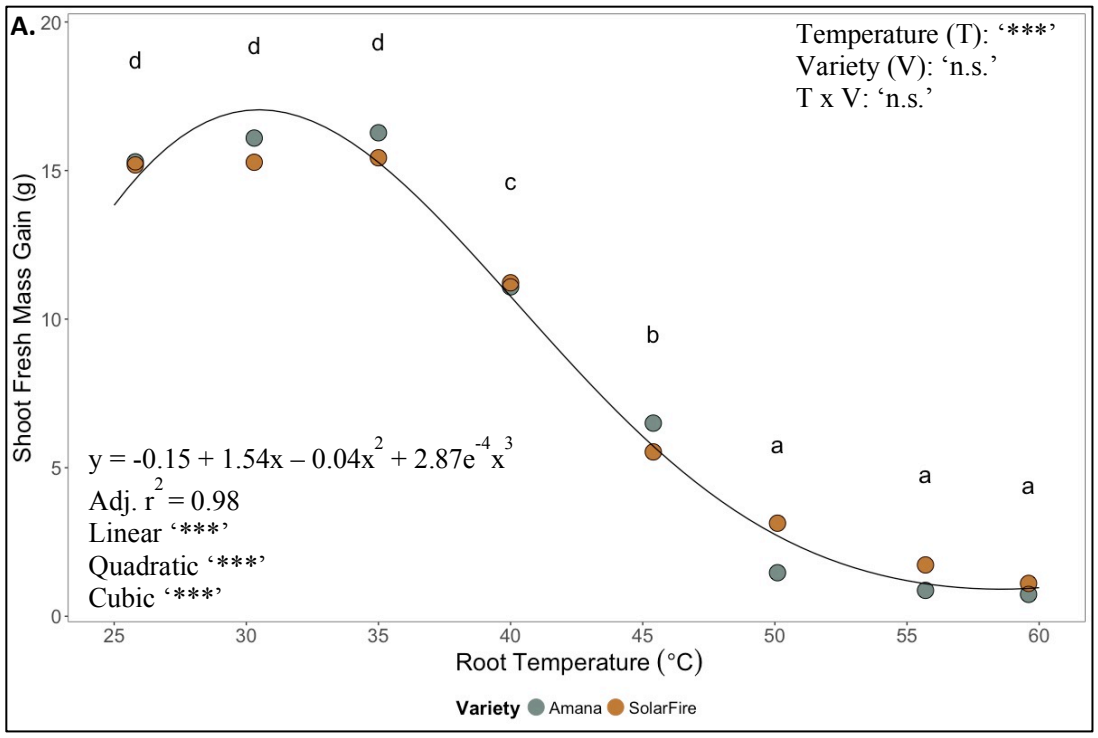
[z] 'n.s.' indicates $p > 0.05$, '*' indicates $p \leq 0.05$, '**' indicates $p \leq 0.01$, '***' indicates $p \leq 0.001$.

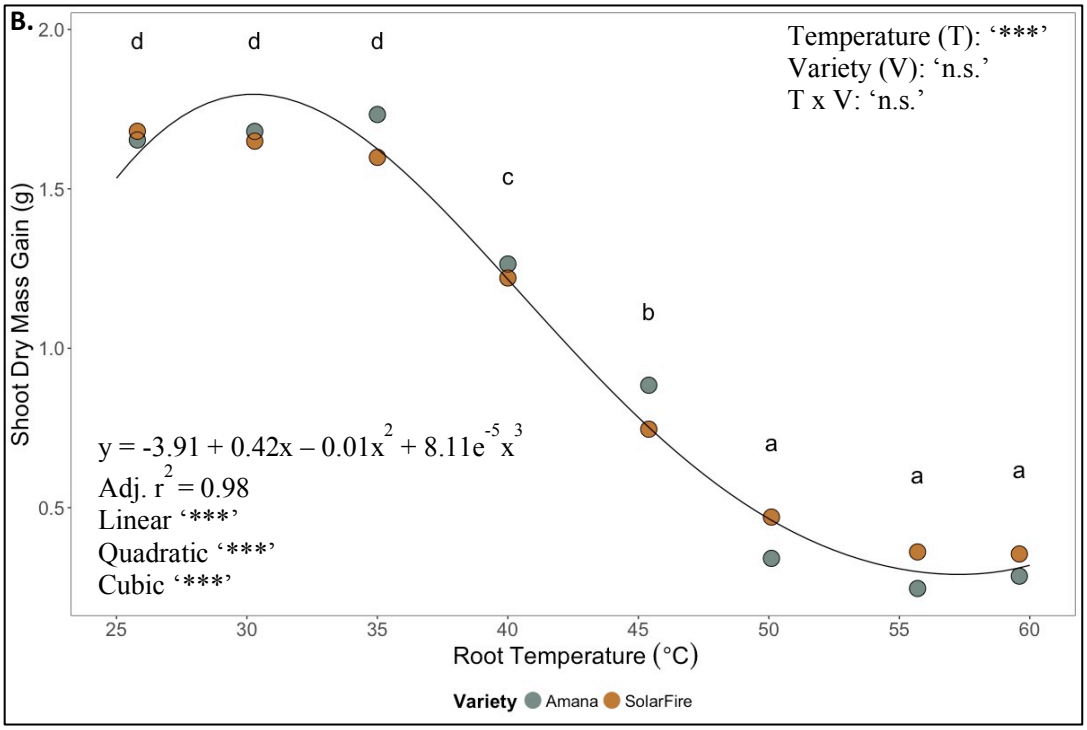
Table 3.2. Root temperature effects on characteristics of growth across the tomato (*Solanum lycopersicum*) varieties 'Amana Orange' and 'Solar Fire'. Letters indicate differences across rows (across RT) determined using Tukey's$_{HSD}$ ($p \leq 0.05$) for mean separation.

Variable	Root Temperature							
	25	30	35	40	45	50	55	60
Stem Height (cm)	13.5 bc	13.9 c	14.0 c	13.8 c	12.3 b	10.2 a	9.3 a	9.3 a
Third Leaf Length (cm)	20.1 c	20.1 c	20.0 c	17.3 c	13.2 b	11.5 ab	9.1 a	10.8 ab
Third Leaf Width (cm)	17.8 d	17.8 d	17.8 d	15.2 c	11.1 b	9.1 ab	7.6 a	7.6 a
Shoot Fresh Mass Gain (g)	15.24 d	15.72 d	15.85 d	11.15 c	6.01 b	2.30 a	1.30 a	0.92 a
Shoot Dry Mass Gain (g)	1.67 d	1.67 d	1.67 d	1.24 c	0.82 b	0.41 a	0.32 a	0.30 a
Root Fresh Mass Gain (g)	4.08 d	4.03 d	4.01 d	2.54 c	0.85 b	0.06 a	-0.12 a	-0.22 a
Root Dry Mass Gain (g)	0.28 d	0.29 d	0.30 d	0.20 c	0.09 b	0.02 a	0.01 a	-0.01 a
Percent Reduction in Shoot Fresh Mass	---	-2.82 a	-3.64 a	24.53 b	55.41 c	77.67 d	83.69 d	85.97 d
Percent Reduction in Shoot Dry Mass	---	-0.01 a	0.04 a	23.67 b	47.48 c	70.33 d	76.01 d	75.10 d
Percent Reduction in Root Fresh Mass	---	2.74 a	1.54 a	34.69 b	72.37 c	90.17 d	94.11 de	96.70 e
Percent Reduction in Root Dry Mass	---	-1.18 a	-4.06 a	26.89 b	62.35 c	84.17 d	89.39 de	93.13 e

Table 3.3. Root temperature and tomato (*Solanum lycopersicum*) variety effects on leaf unfolding (total number of leaves with a petiole angle ≥ 45°). Letters indicate differences across rows (across RT) determined using Tukey's$_{HSD}$ for mean separation ($p \leq 0.05$).

Variety	Root Temperature							
	25	30	35	40	45	50	55	60
Amana Orange	5.4 Bb	5.7 Bb	5.9 Bb	5.3 Bb	5.3 Bb	3.9 Aa	4.0 Aa	3.7 Aa
Solar Fire	4.9 Ac	4.8 Ac	5.0 Ac	4.6 Abc	4.0 Aab	3.9 Aa	4.0 Aab	3.7 Aa


A.
Temperature (T): '***'
Variety (V): 'n.s.'
T x V: 'n.s.'
d
d
d
c
b
a
a
a
y = -0.15 + 1.54x – 0.04x^2 + 2.87e^-4 x^3
Adj. r^2 = 0.98
Linear '***'
Quadratic '***'
Cubic '***'
Shoot Fresh Mass Gain (g)
Root Temperature (°C)
Variety Amana SolarFire


B.
Temperature (T): '***'
Variety (V): 'n.s.'
T x V: 'n.s.'
d
d
d
c
b
a
a
a
y = -3.91 + 0.42x – 0.01x^2 + 8.11e^-5 x^3
Adj. r^2 = 0.98
Linear '***'
Quadratic '***'
Cubic '***'
Shoot Dry Mass Gain (g)
Root Temperature (°C)
Variety Amana SolarFire

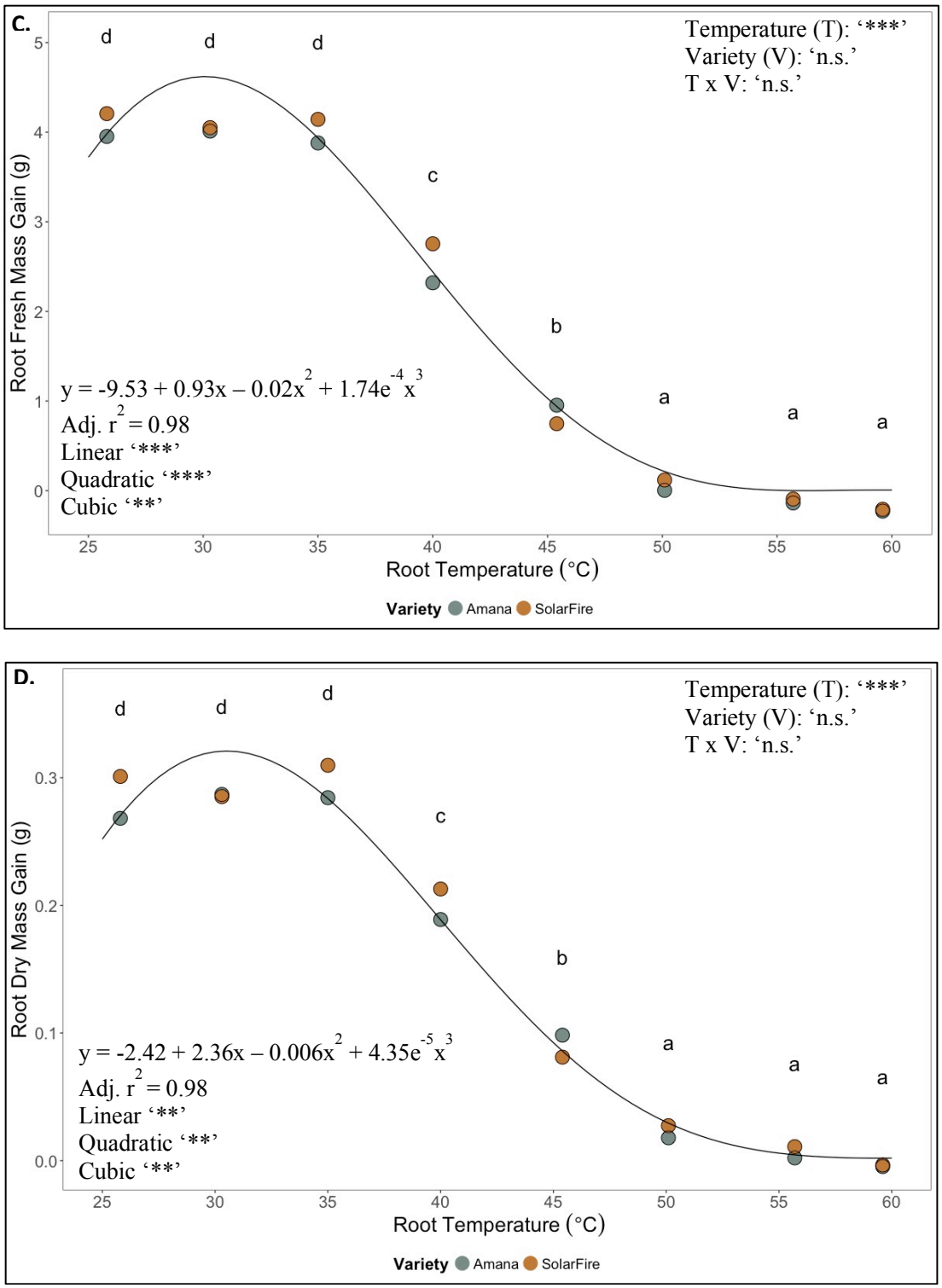
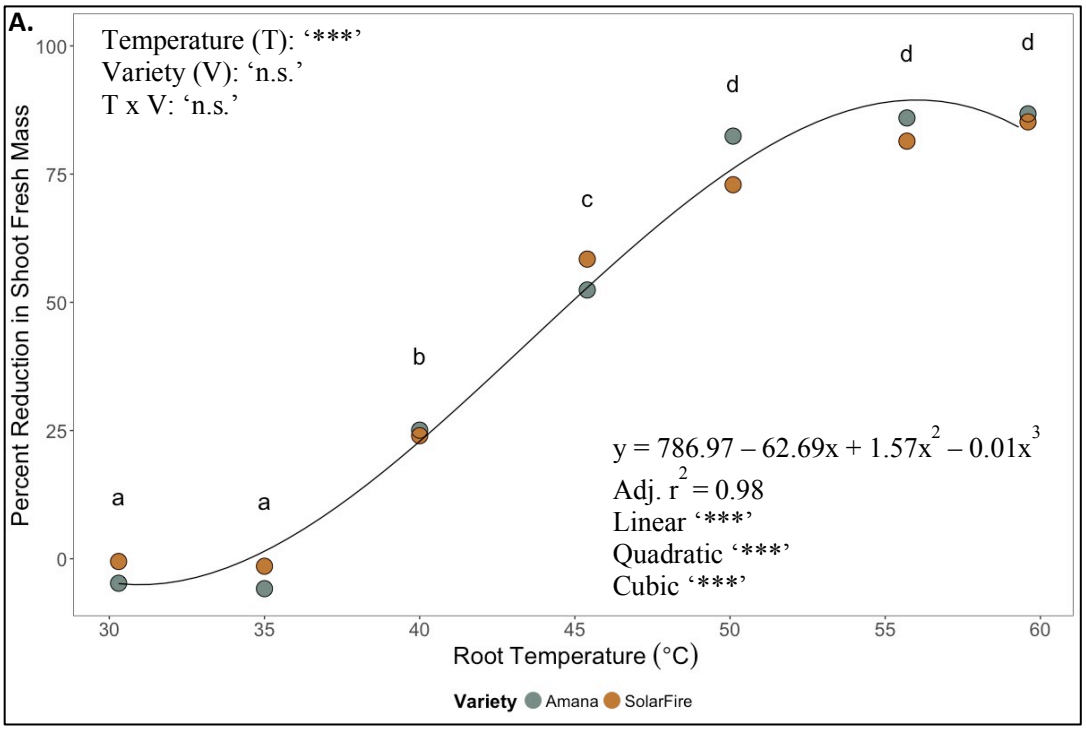
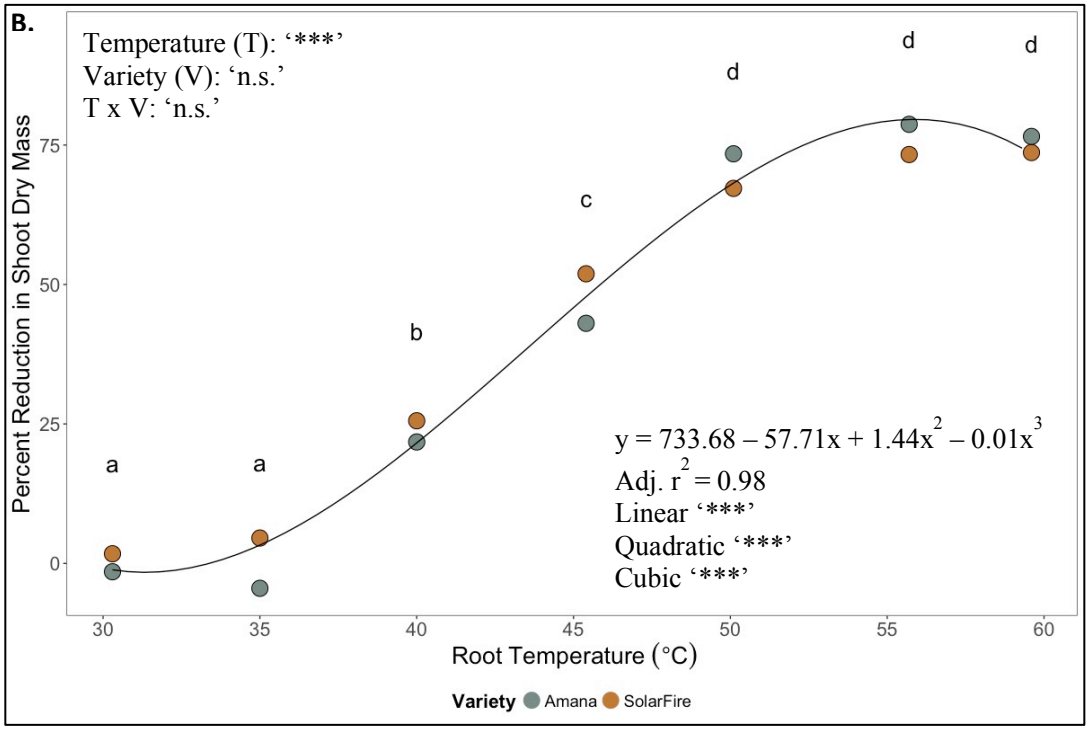


Figure 3.3. Root temperature effects on shoot and root fresh and dry mass gain of the tomato (*Solanum lycopersicum*) varieties 'Amana Orange' (gray-green dots) and 'Solar Fire' (orange dots) grown at high root temperatures for 8 h d^{-1} for 10 d. Letters indicate differences between temperatures determined via Tukey's$_{HSD}$ ($p \leq 0.05$), as no difference between varieties was observed via one-way ANOVA. Analysis of variance is presented with 'n.s.' = $p > 0.05$, '*' = $p \leq 0.05$, '**' = $p \leq 0.01$, '***' = $p \leq 0.001$).


A.
Temperature (T): '***'
Variety (V): 'n.s.'
T x V: 'n.s.'
Percent Reduction in Shoot Fresh Mass
Root Temperature (°C)
y = 786.97 – 62.69x + 1.57x^2 – 0.01x^3
Adj. r^2 = 0.98
Linear '***'
Quadratic '***'
Cubic '***'
Variety Amana SolarFire


B.
Temperature (T): '***'
Variety (V): 'n.s.'
T x V: 'n.s.'
Percent Reduction in Shoot Dry Mass
Root Temperature (°C)
y = 733.68 – 57.71x + 1.44x^2 – 0.01x^3
Adj. r^2 = 0.98
Linear '***'
Quadratic '***'
Cubic '***'
Variety Amana SolarFire

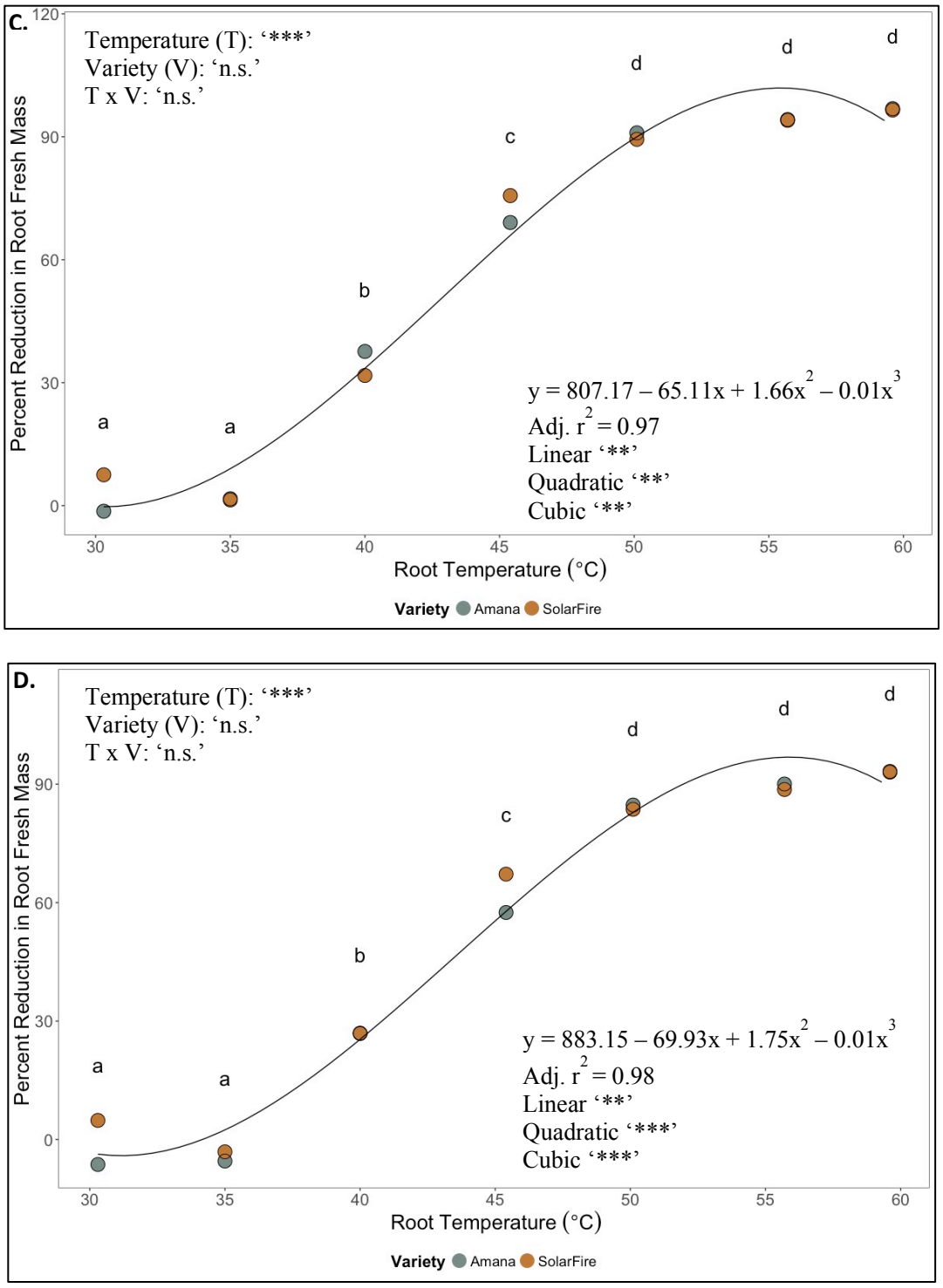
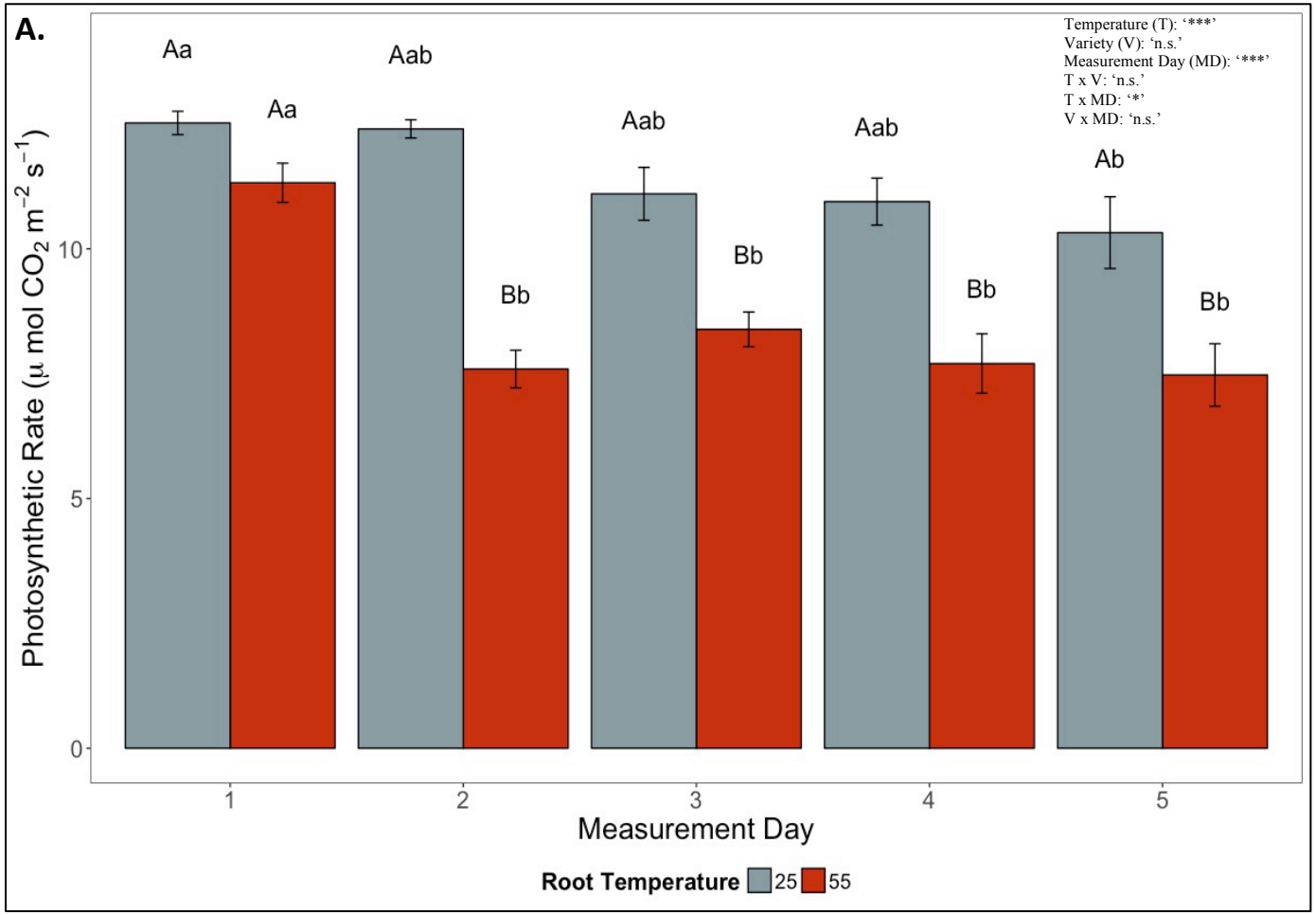


Figure 3.4. Root temperature effects on percent reduction in shoot and root fresh and dry mass gain of the tomato (*Solanum lycopersicum*) varieties 'Amana Orange' (gray-green dots) and 'Solar Fire' (orange dots) grown at high root zone temperatures for 8 h d^{-1} for 10 d. Letters indicate differences between temperatures determined via Tukey's$_{HSD}$ ($p \leq 0.05$), as no difference between varieties was observed via one-way ANOVA. Analysis of variance is presented with 'n.s.' = $p > 0.05$, '*' = $p \leq 0.05$, '**' = $p \leq 0.01$, '***' = $p \leq 0.001$).


A.
Temperature (T): '***'
Variety (V): 'n.s.'
Measurement Day (MD): '***'
T x V: 'n.s.'
T x MD: '*'
V x MD: 'n.s.'
Aa
Aa
Aab
Bb
Aab
Bb
Aab
Bb
Ab
Bb
Photosynthetic Rate (μ mol CO2 m−2 s−1)
0
5
10
1
2
3
4
5
Measurement Day
Root Temperature 25 55

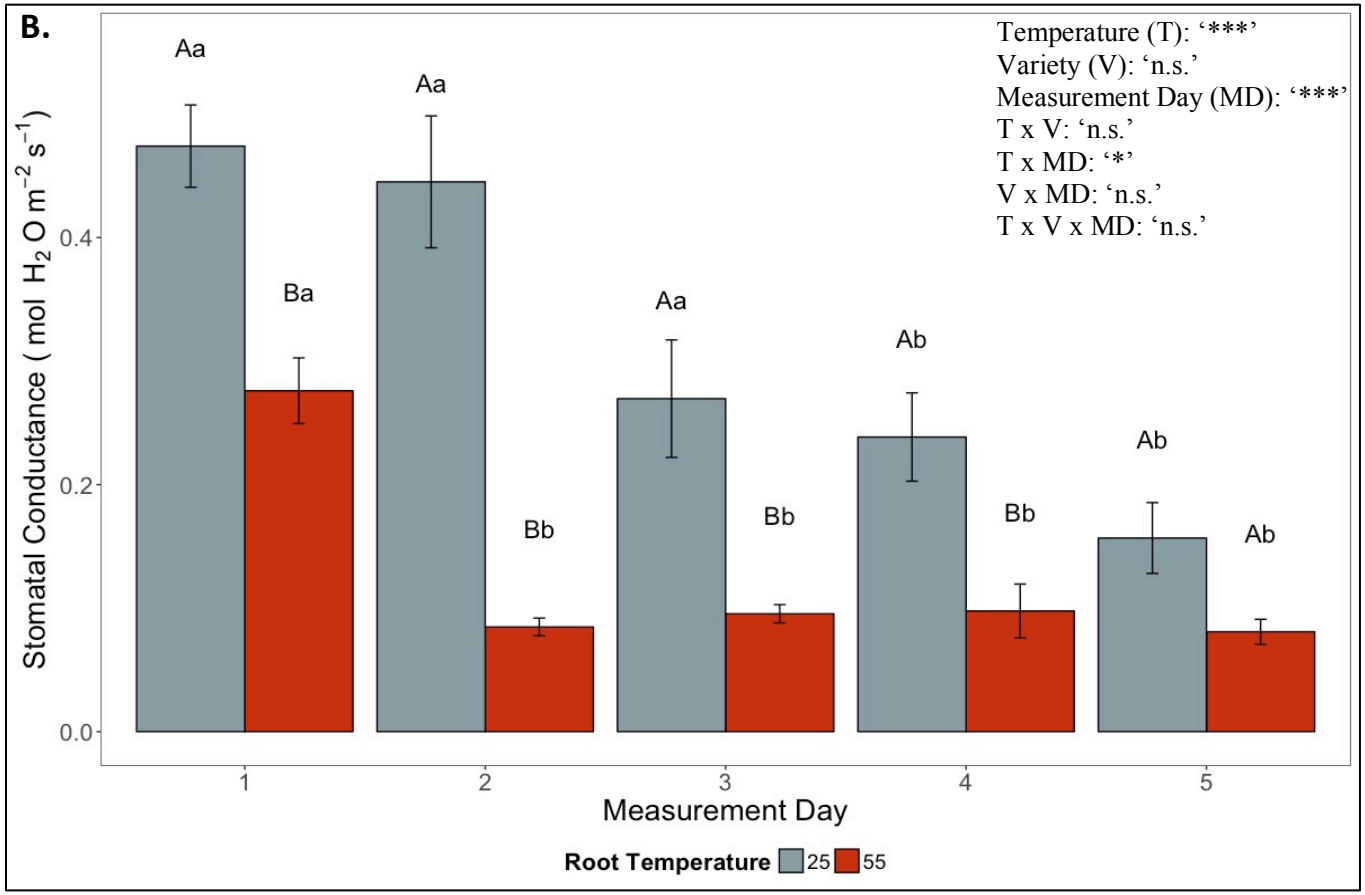
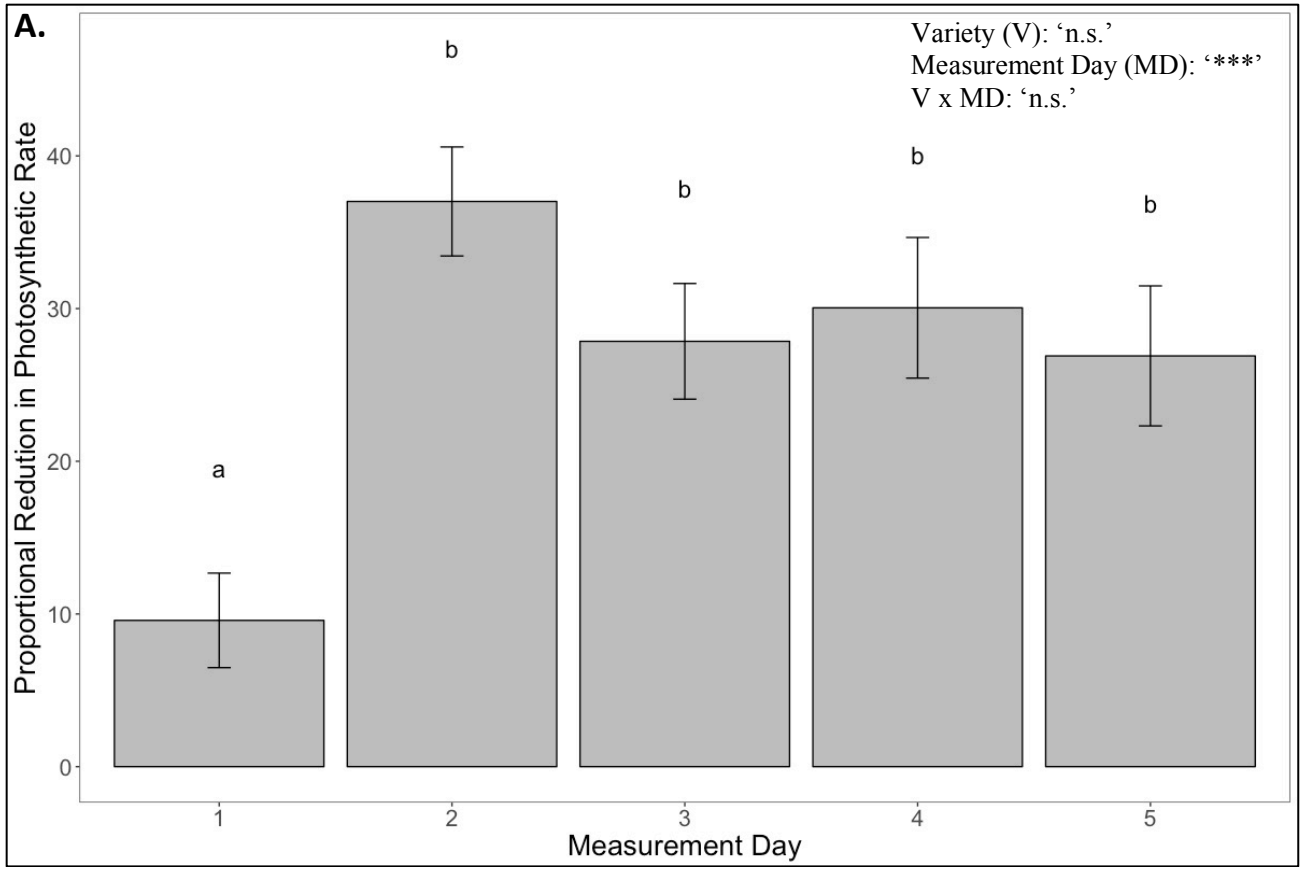


Figure 3.5. Acute high root temperature effects on photosynthetic rate (A) and stomatal conductance (B) of the tomato (*Solanum lycopersicum*) varieties 'Amana Orange' and 'Solar Fire'. Responses were measured during (day 1) a 260 min afternoon exposure to root zone temperatures of 25.0 +/- 0.8°C (gray bars) or 55.5 +/- 0.5°C (red bars). Subsequent measurements on days 2-5 were taken at RT of 25.9 +/- 0.5°C. Letters indicate differences in RT effects (uppercase letters) and measurement day (lowercase letters) across tomato varieties determined via Tukey's s_{HSD} ($p \leq 0.05$) and error bars indicate +/-1 SE. Analysis of variance is presented with 'n.s.' = $p > 0.05$, '*' = $p \leq 0.05$, '**' = $p \leq 0.01$, '***' = $p \leq 0.001$).


A.
Variety (V): 'n.s.'
Measurement Day (MD): '***'
V x MD: 'n.s.'
Proportional Redution in Photosynthetic Rate
0
10
20
30
40
a
b
b
b
b
1
2
3
4
5
Measurement Day

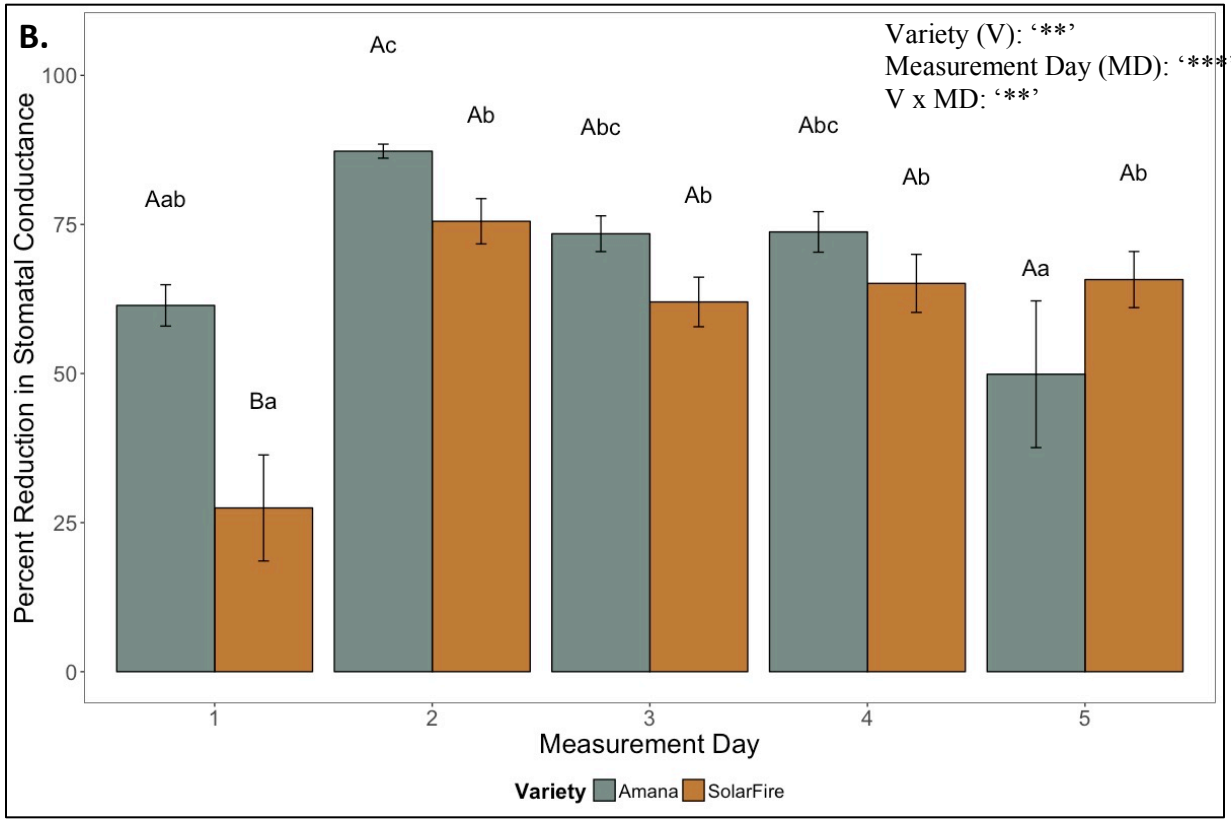


Figure 3.6. Acute high root temperature effects on the daily percent reduction in photosynthetic rate (P_n) and stomatal conductance (g_s) of the tomato (*Solanum lycopersicum*) varieties 'Amana Orange' and 'Solar Fire'. Reductions represent the daily differences between plants exposed to 55.5 +/- 0.5°C root zone temperatures for 260min on day 1 and plants maintained at 25.0 +/- 0.8°C. For percent reduction in P_n (A), letters denote differences between measurement days across varieties and RT. For percent reduction in g_s (B), capital letters denote differences between tomato varieties ('Amana Orange' = gray-green bars, 'Solar Fire' = orange bars) within measurement days, while lowercase letters denote differences between measurement days within varieties. Tukey's$_{HSD}$ was used for mean separation ($p \leq 0.05$) following analysis via one-way ANOVA and error bars indicate +/-1 SE. Analysis of variance is presented with 'n.s.' = $p > 0.05$, '*' = $p \leq 0.05$, '**' = $p \leq 0.01$, '***' = $p \leq 0.001$).

Chapter 4

Microbial inoculant effects on tomato (*Solanum lycopersicum*) growth responses to high root zone temperatures

Container-grown plants can be exposed to high root temperatures (HRT) that can damage root tissue and cause long-term growth reduction. We explored whether detrimental HRT effects on plant growth could be alleviated by applying five root-associated fungi and bacteria (*Azospirillum brasiliense, Bacillus amyloliquifaciens, Curvularia protuberata, Glomus intraradices,* and *Trichoderma harzianum*) thought to confer increased resistance to biotic and abiotic stresses. 'Amana Orange' tomato (*Solanum lycopersicum*) seedlings were inoculated with the beforementioned microbes and exposed to root temperatures between 35 (control) and 55°C (HRT) for 8 h^{-1} d^{-1} over a 10 d period. Plant height and shoot, root, and total plant fresh and dry mass decreased as root temperature increased from 35 to 50ºC. Reduced shoot fresh mass gain occurred with increasing temperature for plants inoculated with all microbes between 45 and 50°C, except for plants treated with *G. intraradices* which had incremental reductions as temperature increased, from 37.77 g at 35°C to 25.31 g at 45°C and 14.72 g at 50°C. Root fresh mass gain was impacted by temperature; decreasing from 3.59 g at 35°C to 2.68 g at 45°C and 0.76 g at 50°C. Dry mass gain of roots and shoots did not differ between uninoculated and inoculated plants, but some differences were observed between inoculant species; for example *G. intraradices*-treated plants accumulated more root dry mass (0.21 g) than those treated with *C. protuberata* (0.17 g), *A. brasiliense* (0.17 g), or *T. harzianum* (0.16 g). Our

results suggested HRT have detrimental effects on above- and below-ground tomato growth and inoculation with the before-mentioned organisms did not alleviate those detrimental effects.

Introduction

Above-ground containerized production of horticultural crops can expose plants to medium and root temperatures (hereafter RT) higher than in the ground. RT as high as 50 to 57°C were observed in sun-exposed containers in Kansas, California, and Minnesota (USA; Lyles et al., 1992; Markham et al., 2011). Previously reported optimal temperatures for root and shoot growth of warm-temperature preferring herbaceous plants such as tomato (*Solanum lycopersicum*) generally range between 25 and 35°C (Hurewitz and Janes, 1983; Klock et al., 1997).

'Heat stress' can reduce plant growth. Wahid et al. (2007) defined 'heat stress' as the response of tissues to temperatures 10-15°C above growing optima. Other research suggested temperature thresholds for heat stress effects on plant growth may be narrower (Lai & He 2016). In either case, the beforementioned observed high container media temperatures could be described as a source of root 'heat stress'. Root heat stress can damage root cell membranes resulting in a loss of internal solutes, limit stomatal conductance and photosynthesis, and/or reduce plant growth in general (Ruter 1993; Du & Tachibana 1994a; Huang & Xu 2000; Nada *et al.* 2003; Benlloch-González *et al.* 2017).

Physical and biological methods have potential to mitigate root high temperature stress effects on plant growth. Container media temperatures in white containers were lower than in green or black containers (Markham *et al.* 2011). Media temperatures in containers made of porous container materials, such as wood pulp and fabric, are also lower than in plastic containers (Nambuthiri *et al.* 2015). Increasingly, microbial inoculants are being studied to determine whether they mitigate abiotic plant stressors such as high temperatures. For instance, *Bacillus licheniformis* 'CH102' improved heat and drought tolerance of *Arabidopsis thaliana* and up-regulating defense pathway-associated transcription factors (Sukkasem *et al.* 2018). Media inoculation with *Septoglomus constrictum* improved heat and drought stress impacts on tomato (*S. lycopersicum* var. 'Moneymaker') shoot and root dry weight, photosystem II photochemical efficiency, and leaf water potential (Duc *et al.* 2018). Canola (*Brassica napus*) seedlings inoculated with *Enterobacter cloacae* 'HSNJ4' had reduced salt-stress induced ethylene production and increased antioxidant activity, associated with bacterial production of ACC-deaminase compared to uninoculated plants (Li *et al.* 2017).

Five inoculant species were identified for this study based upon previous associations with the alleviation of biotic and abiotic contributors to plant stress. Inoculation with thermotolerant fungal endophytes in the genus *Curvularia*, facilitates growth of bunchgrass (*Dichanthelium lanuginosum*) in thermal soils with high temperatures adjacent to geothermal sites in Yellowstone National Park (Redman 2002). Similarly, *ex-situ* inoculation with *C. protuberata* improved survival of tomato plants (*S. lycopersicum* var. 'Rutgers') exposed to 65°C RT for 14 h d^{-1} over14 d (Luis M. Márquez 2007). *Trichoderma harzianum* mitigated osmotic, temperature, and pathogen stress

effects (Mastouri *et al.* 2010; Mona *et al.* 2017). *Glomus intraradices* ameliorated both drought and salinity effects (Hajiboland *et al.* 2010; Ruíz-Sánchez *et al.* 2011; Estrada *et al.* 2013; Scagel *et al.* 2017). *Azospirillum brasiliense* applied alone and in combination with *G. intraradices* ameliorated salt stress and drought stress effects (Ruíz-Sánchez *et al.* 2011; Cohen *et al.* 2015). *Bacillus amyloliquifaciens* inhibited plant pathogens and conferred salt stress tolerance (Chowdhury *et al.* 2013; Liu *et al.* 2017).

In the experiment we determined whether inoculation of tomato (*Solanum lycopersicum* var. 'Amana Orange') seedlings with the aforementioned five root-associated microbes improved root and/or plant growth when exposed to cyclical stressful high RT. We believed that inoculation would improve tomato growth at elevated RT, but that effects would vary between different inoculant species.

Materials and Methods

The tomato (*Solanum lycopersicum*) variety 'Amana Orange' was selected as a model plant because it was characterized as 'heat-sensitive' based on maximum photosystem II quantum efficiency at high air temperatures (Zhou, Yu, *et al.* 2015). 'Amana Orange' seed were obtained from Tomato Grower's Supply (Fort Myers, FL) and were sown into 50-cell trays (one seed per cell; vol. 75 mL; TO Plastics, Clearwater MN) in a soilless media (SunGro SS#8-F2; Agawam, MA) and were lightly covered with vermiculite (3 mm). Seed germination was promoted, and early growth occurred in a greenhouse natural daylight plus 100 μmol m^{-1} s^{-1} supplemental high intensity discharge lighting from 0800 to 0200 HR (18 h d; *mol per day to be determined*), and constant 28.8ºC +/- 1.1°C air temperature. Seed/plants were watered as needed to maintain moist

media and fertilized through the irrigation water with Peters Excel CalMag 15-5-15 (250 ppm N; ICL Specialty Fertilizers; Summerville, SC).

Microbial inoculants with potential to increase high temperature tolerance were selected from the genera *Azospirillum, Bacillus, Curvularia, Glomus,* and *Trichoderma* based on previous studies that demonstrated beneficial effects when plants were exposed to biotic and/or abiotic stresses (Luis M. Márquez 2007; Matsubara *et al.* 2014; Hashem *et al.* 2016; Mona *et al.* 2017; Sukkasem *et al.* 2018). Three commercialized species were obtained in a powdered or liquid-suspended form and were suspended in irrigation water at supplier-recommended application rates for transplanting and/or drenching; *Azospirillum brasiliense* (Azos; Green Diamond Biologicals & Nutritionals, Gilroy, CA) was concentrated to 15.11 colony forming units (cfu)/mL. *Bacillus amyloliquifaciens* (Hydroguard; Botanicare, Chandler, AZ) was applied at a concentration of 5.28 cfu/mL. *Glomus intraradices* (Mykos; Green Diamond Biologicals & Nutritionals, Gilroy, CA) was applied at a concentration of 0.72 propagules (including emulsified spores, hyphae, and colonized root fragments) per mL^{-1}. Spores of two additional microbe species, *Curvularia protuberata* 'Cp4666D' and *Trichoderma harzianum* 'ThTS' (Adaptive Symbiotic Technologies; Seattle, WA) were isolated from agar-based culture media/liquid suspensions and diluted in ammonium phosphate-gel buffer (Kingsley M T & B 1981) to concentrations recommended by the supplier. *C. protuberata* 'Cp4666D' spores were prepared in liquid suspension at a concentration of approximately $1.16x10^4$ spores/mL. *T. harzianum* 'ThTS' was prepared at a liquid concentration of approximately $1.2x10^4$ spores/mL.

Twenty ‘Amana Orange’ seedlings were inoculated with liquid suspensions of each microbial inoculant after two leaves had unfolded (16 d after germination). Inoculant suspensions were agitated between applications to ensure inoculation consistency across replicates. Each seedling was inoculated with 10 mL of a liquid-suspended inoculant injected into media 0.5 cm from the stem using a laboratory-grade pipette. Un-inoculated plants were treated with 10 mL irrigation water only. Following inoculation, the media surface of was covered with 1 cm of the potting medium to prevent desiccation of fresh inoculum.

Inoculated plants were grown in the same greenhouse as pre-inoculation plants for an additional week to allow microbial colonization of root systems. Plants from each treatment group were kept in separate trays and watered independently to prevent cross-contamination. After one week, plants were transferred to a growth chamber (Environmental Growth Chambers; Chagrin Falls, OH) with a 12-hr photoperiod (0500-1700 HR; 420 ± 20 μmol m^{-2} s^{-1}) provided with fluorescent and incandescent lamps (75% and 25% total wattage, respectively). Relative humidity was 50% and air day/night temperatures were 25/20°C respectively. Plants were watered and fertilized as described above.

After one day, plants were transferred to a second growth chamber where roots and media were heated in water baths. Plant roots (still in cells) were inserted into waterproof 50 cell trays 80% submerged in plastic bins (27 L; Rubbermaid, Atlanta GA) containing water heated to target temperatures of 35, 45, 50, or 55°C. The 35°C root temperature was considered as ‘non-stressful’ as maximum tomato growth occurred at media temperatures ranging from 25 to 35°C in a previous study (Guenthner & Erwin,

2019 *in review*). Water was heated using a sous-vide immersion heater and circulator (Gourmia GSV140; Brooklyn, NY). A digital temperature probe (Quartz Digi-Thermo, Traceable Products; Webster TX) was inserted 5 cm deep at the edge of root masses to confirm the desired temperatures were achieved. Root and media temperatures in each of the treatments were 34.7°C ± 0.4°C, 44.9°C ± 0.4°C, 49.9°C ± 0.5°C, and 55.1°C ± 0.5°C, respectively. Growth chamber irradiance was 400 ± 20 μmol m^{-2} s^{-1} (100% fluorescent lamps). Leaf temperatures was measured on the second newest unfolded leaf using an infrared radiometer (MI-210; Apogee Instruments, Logan UT) and were 25.1°C ± 0.9°C, 26.7°C ± 0.8°C, 28.6°C ± 0.9°C, and 28.3°C ± 0.6°C, respectively amongst root temperature treatments.

Plants remained in treatment baths for 8 h^{-1} d^{-1} (from 0900-1700 HR) for 10 d. Each morning, plants from each inoculant treatment group were randomly redistributed amongst designated cells (same inoculant) in temperature treatment baths to eliminate edge effects. Daily, at the end of each RT treatment, plants were returned to the first growth chamber for a 12 hr night at 20°C. Plants from each inoculant treatment group were watered and fertilized as described above in separate trays to avoid cross-inoculation.

After the 10 d RT treatments, plants were destructively sampled. Data were collected on unfolded leaf number (leaves with petioles ≥ 45°), stem length, and whether leaf edge browning was apparent. Shoots were excised from roots and weighed for fresh mass. Roots were washed free of media, lightly patted dry with paper towels, and also measured for fresh mass. Shoot and root samples were dried in an oven (Hotpack, Philadelphia PA) at 65°C for 12 d and dry mass of each was determined. Un-inoculated

plants grown alongside treatment plants were also destructively sampled and processed at the start of the 10 d treatment period to determine fresh and dry mass gain among RT treated plants. Treatment effects were evaluated by determining the difference in total mass between un-inoculated plants sampled prior to the start of the RT treatments and plants inoculated and placed in RT treatments for 10 d (hereafter presented as the mass gain). Individual shoot and root mass values within each inoculant treatment group at 45°C and higher RT were divided by the average mass of plants grown at 35°C to determine percent difference in total mass between un-stressed (35°C RT) and stressed (45-55°C RT) plants.

This experiment was organized in a completely randomized statistical design in a factorial arrangement. Inoculant (5 types + un-inoculated) and RT (4 levels) served as the main effects (6 x 4 =24 treatments). There were five replicates for each treatment combination which resulted in a total of 120 plants used in this study. Outliers were identified using the SPSS 'Explore' function and were removed when identified (data which lay outside the interquartile range by three times its inner range. Analysis of percent reductions in mass between treatments was performed after arcsine transformation. All data were processed and analyzed using the SPSS Statistics v. 24 statistical package (IBM Co.; Armonk NY). Tukey's$_{HSD}$ was used for mean separation. LSD was used for mean separation when Tukey's HSD did not discern analysis of variance identified significant effects among means.

Results

Differences in appearance among plants subjected to 35-45°C RT versus 50-55°C RT were apparent by the end of the RT treatments. Plants with RT of 50 and 55°C were smaller than those grown at lower RT (Fig. 4.1). Amongst un-inoculated plants, leaf edge browning was present on plants exposed to 50 and 55°C RT. Browning was present on the lower leaf edges of plants at 45°C RT and higher, though the proportion of plants with browning was only associated with the effect of RT and not inoculant treatment ($p < 0.001$ and p = n.s., respectively). Visual differences in root density were also apparent when RT increased above 35°C, with roots clustering near the upper portion of the plug at RT of 45°C and visually absent at RT of 50 and 55°C (Fig. 4.1).

Plant height was impacted by an interaction between RT and inoculant treatment ($p \leq 0.05$) (Table 4.1). Plant height decreased as RT increased in the absence of inoculants: height of un-inoculated plants was 23.0 cm at 35°C and 20.1-18.1 cm at RT of 45-55°C (Table 4.2). Plant height of inoculated plants, with the exception of those treated with *G. intraradices,* decreased when temperature increased from 45°C to 50°C (Table 4.2); for instance, plant height of *B. amyloliquifaciens* inoculated plants was 23.8 cm at 45°C to 16.8 cm at 50°C (Table 4.2). In contrast, plants treated with *G. intraradices* differed only in stem height at RT of 35°C (23.6 cm) and 50°C (19.2 cm).

RT and inoculants interacted ($p \leq 0.05$) to affect shoot fresh weight gain (Table 4.1). With un-inoculated plants, and those inoculated with *G. intraradices,* shoot fresh mass gain decreased as RT increased from 35°C RT to 45°C, and was lowest at 50 and 55°C (Table 4.2). Plants that received other inoculants had higher shoot fresh mass gain

with 35 and 45°C RT than at 50 and 55°C RT (Table 4.2). RT impacted the percent reduction in shoot fresh mass (Table 4.2); the percent reduction in shoot mass increased from 21% at 45°C to 52% at 50°C RT (Fig. 4.2J).

Independent effects of RT ($p \leq 0.001$) and inoculant treatment ($p \leq 0.01$) affected shoot dry mass gain (Table 4.1). Shoot dry mass gain was higher on plants grown with 35-45°C RT (2.44-2.22 g) than plants grown with a 50-55°C RT (1.33-1.54 g) across inoculants (Fig. 4.2A). Shoot dry mass gain across RT was higher on plants inoculated with *G. intraradices* (2.13 g) than inoculated with *Curvularia protuberata* (1.66 g; Table 4.3).

Percent reduction in shoot dry mass was impacted by RT ($p \leq 0.001$), but not inoculants (Table 4.1), and increased when RT increased above 45°C (Fig. 4.2B). Percent reduction in shoot dry mass was 10% with a RT of 45°C but increased to 31-38% at 50-55°C (Fig. 4.2B).

Root fresh mass gain and percent reduction in root fresh mass were influenced by RT ($p \leq 0.001$ in both cases) but not inoculants (Table 4.1). Root fresh weight gain was higher at a 35°C RT (3.6 g) than at 50-55°C (0.3-0.8 g) across inoculants (Fig. 4.2C). The percent reduction in root fresh mass was least (19%) when grown with 45°C RT and greatest (59-68%) when grown with 50-55°C RT (Fig. 4.2D).

Root dry mass gain was independently impacted by RT ($p \leq 0.001$) and inoculants ($p \leq 0.05$) (Table 4.1). Root dry mass gain decreased from 0.3 g at 35 and 45°C to 0.1g at 50 and 55°C (Fig. 4.2E). Root dry mass gain across inoculants was less than 0g above a 55°C RT. Root dry mass gain was higher across RT on plants inoculated with *G. intraradices* (0.2 g) than those inoculated with *C. protuberata* (0.2 g),

A. brasiliense (0.8 g), or *T. harzianum* (0.8 g), but did not differ from un-inoculated plants (0.2 g) or plants inoculated with *B. amyloliquifaciens* (0.2 g). Percent reduction in root dry mass was only impacted by RT (Table 4.1); increasing from 5% at 45°C to 57% at 55°C (Fig. 4.2F).

Total plant fresh mass gain was impacted by an interaction between RT and inoculant ($p \leq 0.05$) (Table 4.1). Total fresh mass gain across RT was lower for plants treated with *G. intraradices* (10.2 g) and *C. protuberata* (20.0 g) than un-inoculated plants (24.5 g) (Table 4.3). Total fresh mass gain across inoculants decreased from 35°C (36.6 g) to 45°C (27.7 g) and finally 50 and 55°C (13.7 g and 11.4 g, respectively)(Fig. 4.2G). Percent reduction in total fresh mass was impacted by an interaction ($p \leq 0.05$) between RT and inoculant type (Table 4.1). *G. intraradices*-inoculated plants had lower total dry mass gain (1.8 g) than un-inoculated plants (2.3 g), but did not differ from plants that received other inoculants across RT (Table 4.3). Total plant dry mass gain was impacted independently RT ($p \leq 0.001$) and inoculant ($p \leq 0.05$) (Table 4.1). Total dry mass gain across inoculants decreased between 45°C (2.5 g) and 50°C (1.7 g)(Fig. 4.2H). Percent reduction in total dry mass was impacted by RT ($p \leq 0.001$) and inoculants ($p \leq 0.05$) independently (Table 4.1). *B. amyloliquifaciens*-inoculated plants had a greater percent reduction in dry mass across RT than other inoculants (Table 4.3); percent reduction in total dry mass increased from 10% at 45°C to 34-42% at 50-55°C RT (Fig. 4.2I).

Discussion

Results indicated exposure of 'Amana Orange' tomato roots to daily high RT reduced root and shoot mass, regardless of whether plants were inoculated with bacterial or fungal organisms. Our data agree with previous work that showed high RT reduced shoot and root mass. For instance, Hurewitz and Janes (1983) reported RT over 32.2°C for 14d reduced tomato 'Vendor' fresh and dry mass. Tomato 'Jest Star' shoot dry mass was also lower after 19d of growth with a 36°C RT (Klock *et al.* 1997). Data presented here also agree with previous work we conducted with RT varying from 25-60°C on uninoculated plants, wherein RT over 35°C limited shoot and root mass gain (Guenthner and Erwin, 2019 *in review*).

Plant age may have played a role in differences in observed RT stress effects on growth. In our previous study, 'Amana Orange' plants grown at 45°C for 10d had lower root and shoot dry weight gain compared to plants growth at RT of 25-35°C (Guenthner and Erwin, *under review*). In this study, non-inoculated plants did not differ in shoot and root mass dry weight gain between 35 and 45°C. However, high RT were initiated in this study when 4-5 leaves had unfolded rather than 2 leaves as in the previous study, suggesting that older plants may be less responsive to high RT than younger plants. Further exploration of this age-dependent response to high RT would, therefore, be important.

Reduced fresh/dry mass may be due to stress-induced reduction in photosynthesis or tissue damage associated with increased reactive oxygen species (ROS) concentrations, or membrane damage resulting in loss of solutes in heat-stressed tissues. Reduced photosynthesis was associated with reductions in cucumber

(*Cucumis sativus*) whole plant, root, and leaf fresh weight grown at a 38°C RT for 10d (Nada *et al.* 2003). ROS buildup in *Jatropha curcas* roots was higher on roots exposed to 42°C for 12hr than roots maintained at 27°C (Silva *et al.* 2017). Exposure of roots of two Cucurbit species (*Cucurbita ficifolia* and *C. maxima*) for 7d to a 34°C RT increased root ROS (hydrogen peroxide) concentration more than in plants grown at 14 or 24°C RT (Zhang *et al.* 2007). Stimulation of antioxidant activity by beneficial microbes increases abiotic stress resistance in host plants. For example, increased leaf and root antioxidant activity were associated with inoculation of heat and drought-stressed tomatoes with *Septoglomus constrictum* (Duc *et al.* 2018). The presence of *Glomus sp.* fungi was associated with a reduction in root membrane permeability and malondialdehyde (MDA) content in maize (*Zea mays* 'Zhengdan 958') and increased tissue antioxidant concentrations in cyclamen (*Cyclamen persicum* 'Pastel') exposed to supraoptimal air temperatures (Zhu *et al.* 2010; Maya & Matsubara 2013). While metabolic and cellular responses were not evaluated in this study, quantification in future research may shed light on the basis for variation in responses to the high RTs and potential interactions with different microbial species.

Observed reductions in root and shoot growth despite the presence of different inoculants here reflects trends observed in other studies of microbial alleviation of abiotic stress. Reductions in growth on plants exposed to an abiotic stress still occurred (compared to unstressed plants) following inoculation with beneficial microbes, though stressed inoculated plants performed better than un-inoculated plants. For example, treatment of tomatoes ('Zhongzha105') with *Glomus mosseae* improved root and leaf dry weight and fruit weight compared to un-inoculated plants grown with high soil salt

concentrations; yet root and leaf dry weight were still lower than those of unstressed inoculated and un-inoculated plants (Abdel Latef & Chaoxing 2011). Similar improvements in tomato ‘ Moneymaker’ root and shoot dry weight were observed under drought and combined drought and heat stress when media was inoculated with *Septoglomus constrictum* compared to un-inoculated plants (Duc *et al.* 2018).

RT and inoculants interacted to affect plant height and above-media fresh weight (Table 2). Plants treated with all inoculants, with the exception of *G. intraradices*, had similar shoot fresh mass gain between 35 and 45°C RT; *G. intraradices*-inoculated and un-inoculated plant shoot fresh mass gain decreased between 35 and 45°C. Marginal impacts of inoculants were observed on shoot dry mass gain and whole plant fresh and dry mass gain across RT (Table 3). For example, plants treated with *G. intraradices* had higher shoot dry mass gain across temperatures than those treated with *C. protuberata* ‘Cp4666D’ or *T. harzianum* ‘ThTS’. Similarly, plants treated with *G. intraradices* had higher root dry weight gain than those treated with three other inoculants. However, total plant fresh and dry mass gain of *G. intraradices*-inoculated plants was lower than un-inoculated plants and did not otherwise differ from any other inoculants. Side-by-side comparisons of the effects of inoculants on root and whole plant growth responses have not previously been reported. However, differences between the effectiveness of different inoculants were observed under other abiotic stress conditions. For example, inoculation with *A. brasiliense* and *G. intraradices* resulted in different effects on shoot and root fresh weight gain of drought-stressed rice (*Oryza sativa* v. ‘INCA LP-5’) (Ruíz-Sánchez *et al.* 2011).

The lack of differences in root fresh and dry mass gain between plants treated with different inoculants at high RT may indicate either microbial species used here were unable to compensate for the effects of high RT or that application rates were too low or colonization time was inadequate to insure full colonization, or simply because they were ineffective. Alternatively, the high RT may have killed some inoculants negating any beneficial effects. The site of origin for microbe species can also impact survival and effectiveness as beneficial inoculants. For example, a strain of *G. intraradices* isolated from dry, saline soils performed better than a model strain of the same species under saline *in-vitro* conditions, and subsequently better buffered maize (*Zea mays*) against high salinity growing conditions (Estrada *et al.* 2013). This study did make use of a thermotolerant species, *C. protuberata* 'Cp4666D', but this characteristic was not observed to impact the inoculant's effect on tomato growth. Future research determining the extent of colonization may help to shed light on whether or not the lack of observed inoculant effects on root mass were due in part to low inoculant establishment in root masses.

Root colonization by bacteria and arbuscular mycorrhizal fungi (AMF) also varies between species and media conditions. Rate of colonization of pepper roots varied between *Glomus sp.* at temperatures between 32 and 38°C (Martin & Stutz 2004). Likewise, colonization rate of basil (*Ocimum basilicum* v. 'Siam Queen') roots by *G. intraradices* decreased with media salinity, although the presence of this AMF still improved stomatal conductance and shoot fresh weight (Scagel *et al.* 2017). The manufacturer suggested inoculation dosages used in our study presented in this paper for *B. amyloliquifaciens, A. brasiliense,* and *G. intraradices* were magnitudes lower than that

used for *C. protuberata* and *T. harzianum.* Repeated applications of the three aforementioned species are suggested by the manufacturers, and whether or not the single application (used in this study) was enough to colonize roots is uncertain. However, *G. intraradices*-inoculated plants did show inoculant-associated effects (Table 3), suggesting some colonization had occurred. It is possible, especially with the low application dosages of several species, that the seven-day post-inoculation period was not long enough to fully colonize roots. Past studies have made use of different inoculation strategies. For example, Ruiz-Sanchez et al. (2011) inoculated rice (*O. sativa* 'Inca-LP5') with *G. intraradices* once at the time of seed germination and later when plants were transplanted prior to drought stress treatments, *A. brasiliense* was applied at the time of transplanting and again 15d afterwards. Tomatoes (var. 'Rio Grande') treated with *T. harzianum* were inoculated 10 wk prior to the initiation of drought stress conditions (Mona *et al.* 2017). Taken together, an additional study evaluating the impact of increased colonization times and abiotic stress (high RT) tolerance would be useful for comparison.

Conclusion

Our study represents an early foray into the potential use of microbial inoculants for the alleviation of high RT stress on container-grown herbaceous crops. The negative effects of cyclical high RT exposure on tomato roots were evident in this study regardless of the presence of different microbe species. While plant characteristics of aboveground growth in terms of plant fresh mass varied with inoculant species and RT, differences in both aboveground and belowground growth in terms of dry weight did not evidence

microbe-specific impacts on plant performance at high RT. Future exploration of the relationship between these select inoculant species and high RT stress alleviation can be greatly improved through evaluation of both microbial activity (colonization rates etc.) and plant cellular and molecular responses.

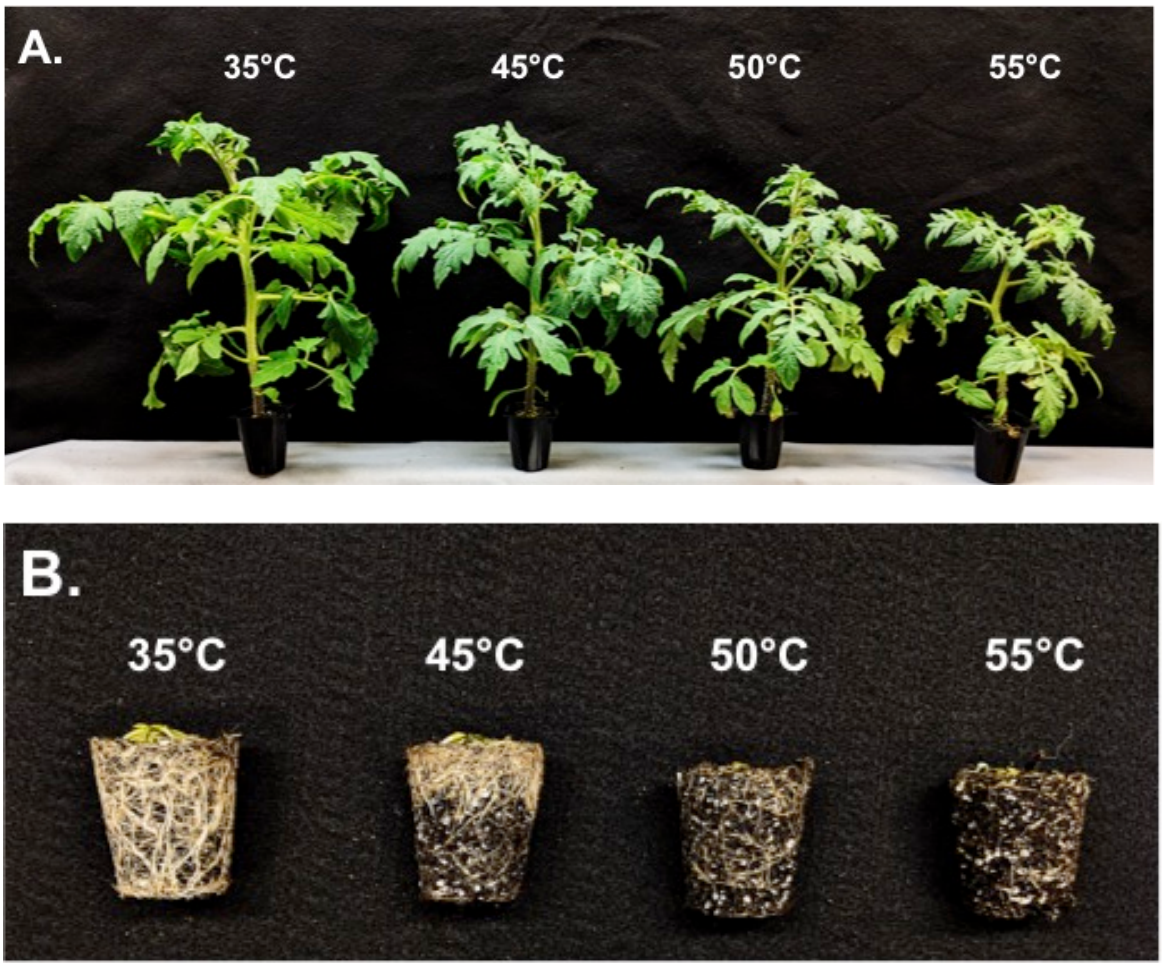


Figure 4.1. Representative images of the shoots (A) and roots (B) of tomatoes (*Solanum lycopersicum* 'Amana Orange') grown at 35, 45, 50, and 55°C root temperatures for 8 h d^{-1} over a 10 d period.

Table 4.1. Analysis of variance for the effects of root temperature and microbial inoculants on tomatoes (*Solanum lycopersicum* 'Amana Orange') grown at root zone temperatures of 35 to 55°C for 8 h d^{-1} for 10 d.

Variable	Factor		
	Temperature	Inoculum	Temperature x Inoculum
Stem Height (cm)	*** [z]	***	*
Shoot Fresh Mass Gain (g)	***	***	*
Percent Reduction in Shoot Fresh Mass	***	n.s.	n.s.
Root Fresh Mass Gain (g)	***	n.s.	n.s.
Percent Reduction in Root Fresh Mass	***	n.s.	n.s.
Total Plant Fresh Mass Gain (g)	***	**	*
Percent Reduction in Total Fresh Mass	***	***	*
Shoot Dry Mass Gain (g)	***	**	n.s.
Percent Reduction in Shoot Dry Mass	***	n.s.	n.s.
Root Dry Mass Gain (g)	***	*	n.s.
Percent Reduction in Root Dry Mass	***	n.s.	n.s.
Total Plant Dry Mass Gain (g)	***	*	n.s.
Percent Reduction in Total Dry Mass	***	**	n.s.

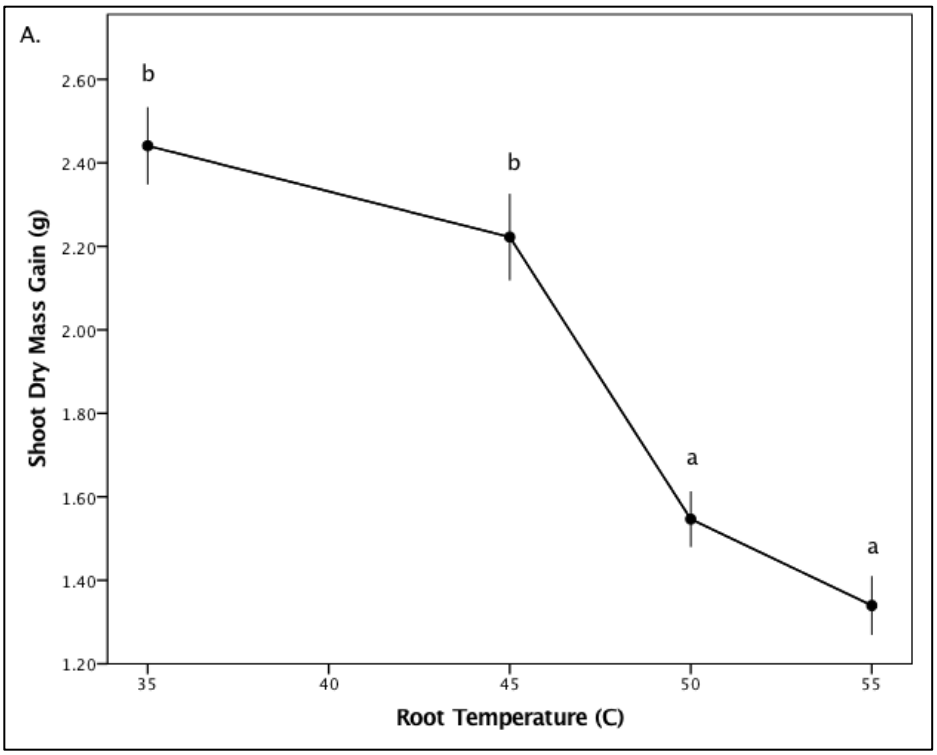
[z] 'n.s.' indicates $p > 0.05$, '*' indicates $p \leq 0.05$, '**' indicates $p \leq 0.01$, '***' indicates $p \leq 0.001$.

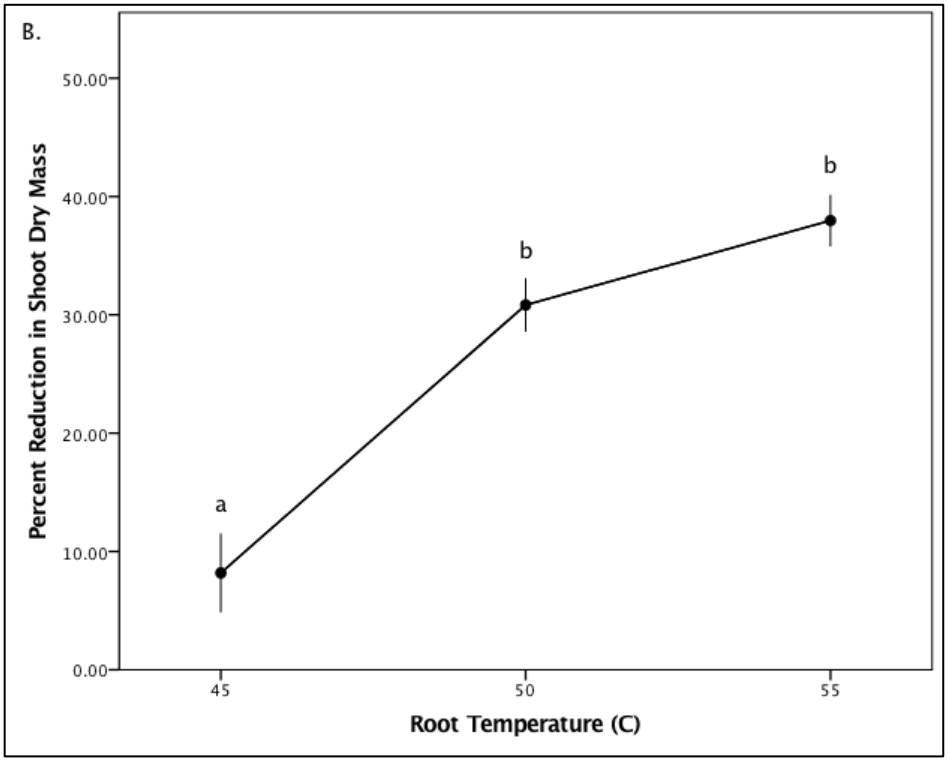
Table 4.2. Interactive effects of root temperature and microbial inoculant species on growth characteristics of the tomato (*Solanum lycopersicum*) variety 'Amana Orange'. Plants were grown at HRT for 8 h d^{-1} over a 10 d period. Letters indicate differences within characteristics across all inoculant types determined using Tukey's$_{HSD}$ for mean separation following analysis via two-way ANOVA. Capital letters going down show the interaction within RT, while lowercase letters show the interaction within inoculants across RT.

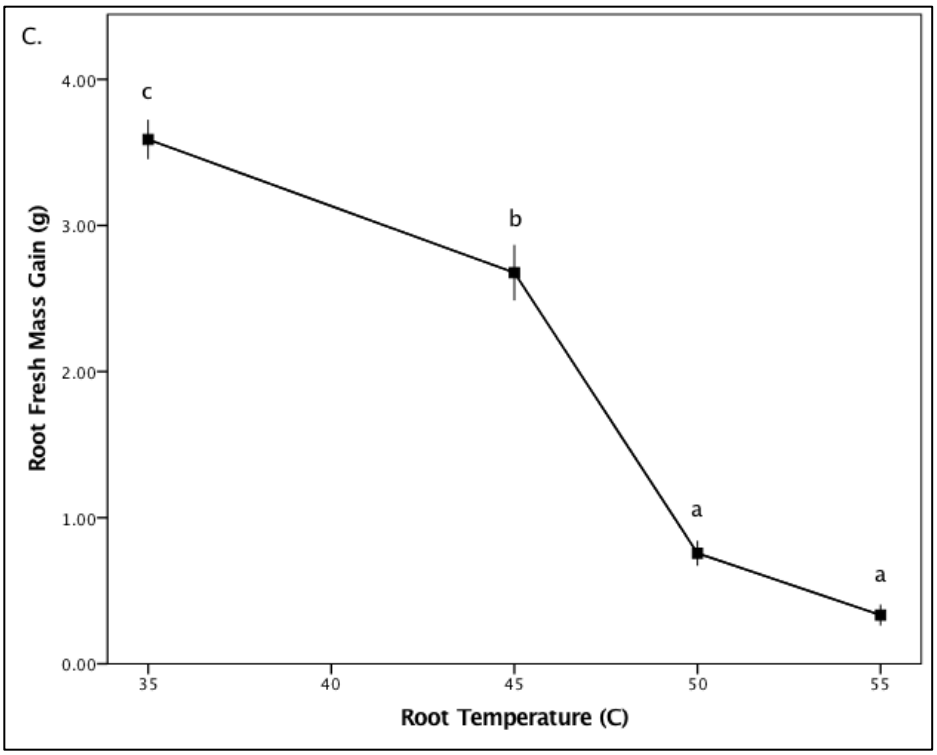
Inoculant/Characteristic	Root Temperature (°C)			
	35	45	50	55
None (Un-Inoculated)				
- Plant Height (cm)	23.0 Ab	20.1 Aa	18.06 Aa	19.02 Aa
- Shoot Fresh Mass Gain (g)	33.98 Ac	22.45 Ab	14.64 Aa	12.13 ABa
- % Reduction in Shoot Fresh Mass	---	28.70 Aa	48.24 Ab	54.52 Ab
- % Reduction in Total Fresh Mass	---	28.40 Aa	53.34 Ab	53.68 Ab
Azospirillum brasiliense				
- Plant Height (cm)	23.8 Ab	23.6 Bb	19.0 Aa	17.5 Aa
- Shoot Fresh Mass Gain (g)	34.05 Ab	27.84 Ab	13.28 Aa	9.77 ABa
- % Reduction in Shoot Fresh Mass	---	15.43 Aa	51.72 Ab	60.48 Ab
- % Reduction in Total Fresh Mass	---	28.24 Aa	49.76 Ab	55.76 Ab
Bacillus amyloliquifaciens				
- Plant Height (cm)	22.8 Ab	23.8 Bb	16.82 Aa	17.7 Aa
- Shoot Fresh Mass Gain (g)	34.71 Ab	30.95 Ab	11.46 Aa	9.25 Aa
- % Reduction in Shoot Fresh Mass	---	9.18 Aa	56.94 Ab	62.38 Ab
- % Reduction in Total Fresh Mass	---	26.92 Aa	68.94 Bb	78.04 Bb
Curvularia protuberata				
- Plant Height (cm)	22.9 Ab	20.8 ABb	17.3 Aa	17.0 Aa
- Shoot Fresh Mass Gain (g)	30.69 Ab	23.24 Ab	9.69 Aa	10.18 ABa
- % Reduction in Shoot Fresh Mass	---	20.26 Aa	57.08 Ab	55.72 Ab
- % Reduction in Total Fresh Mass	---	16.88 Aa	58.03 ABb	63.94 Ab
Glomus intraradices				
- Plant Height (cm)	23.6 Ab	22.0 ABab	19.2 Aa	20.5 Aab
- Shoot Fresh Mass Gain (g)	37.77 Ac	25.31 Ab	14.72 Aa	15.07 Ba
- % Reduction in Shoot Fresh Mass	---	28.36 Aa	52.52 Ab	51.74 Ab
- % Reduction in Total Fresh Mass	---	19.70 Aa	56.93 ABb	56.19 Ab
Trichoderma harzianum				
- Plant Height (cm)	21.9 Ab	20.7 ABb	17.5 Aa	17.7 Aa
- Shoot Fresh Mass Gain (g)	29.10 Ab	22.23 Ab	13.88 Aa	9.86 ABa
- % Reduction in Shoot Fresh Mass	---	19.50 Aa	43.24 Ab	54.66 Ab
- % Reduction in Total Fresh Mass	---	20.08 Aa	44.49 Ab	56.12 Ab

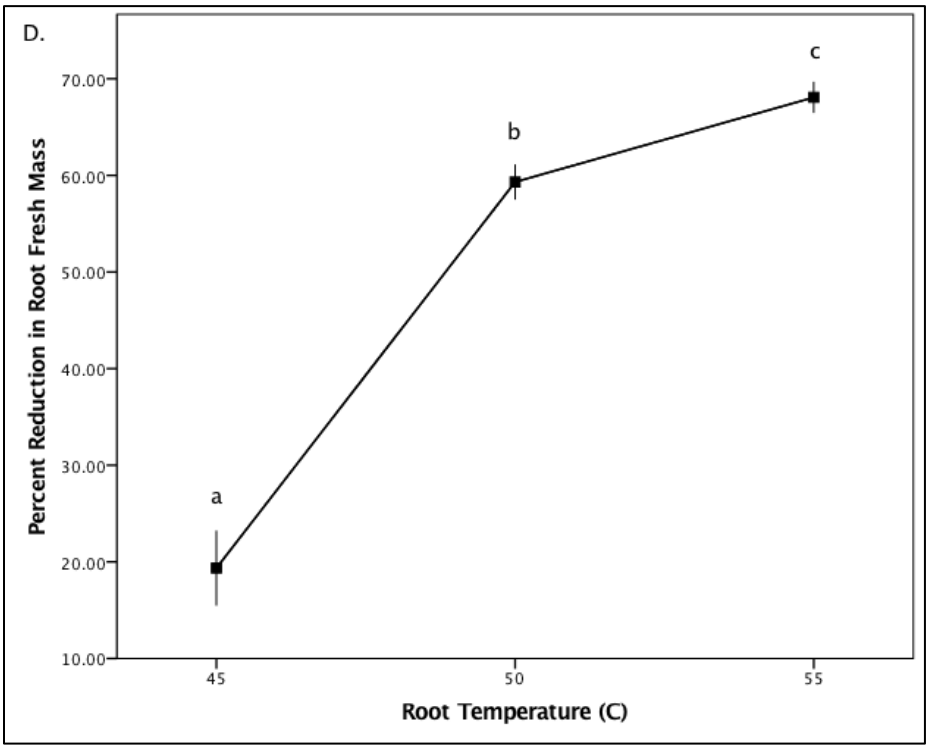
Table 4.3. Microbial inoculant species effects on shoot, root, and total fresh and dry mass gain and percent reduction in total dry mass from 35°C of the tomato (*Solanum lycopersicum*) variety 'Amana Orange' across RT. Plants were grown at RT of 35, 45, 50, and 55°C for 8 h d^{-1} over a 10 d period. Letters indicate differences within characteristics (down columns) determined using Tukey's $_{HSD}$ for mean separation ($p \leq 0.05$) of shoot dry weight gain and LSD for mean separation ($p \leq 0.05$) of root dry weight gain following analysis via one-way ANOVA.

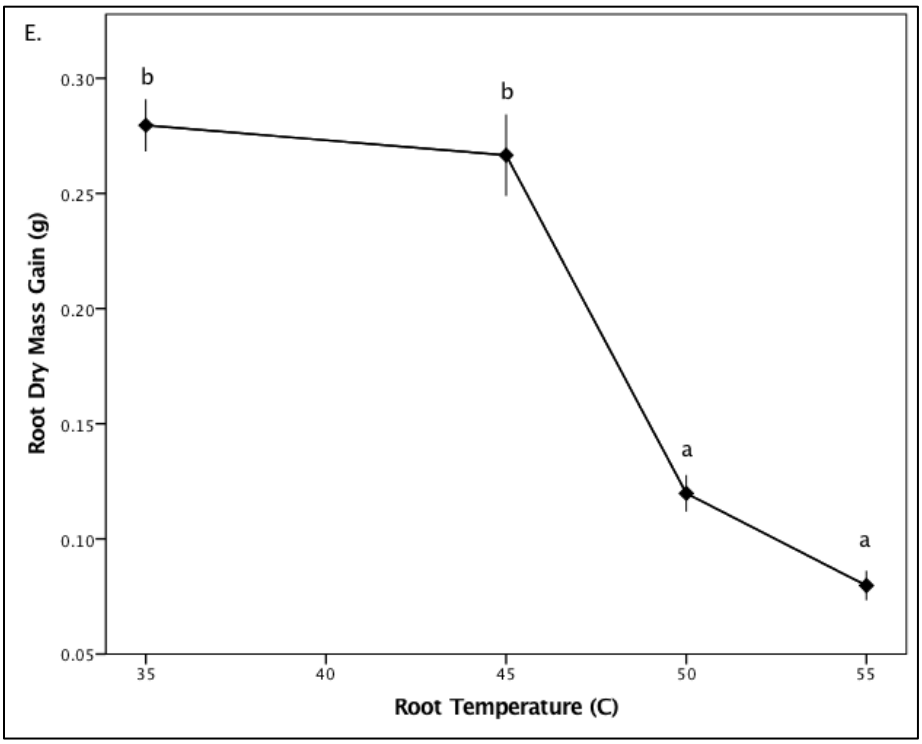
Inoculant Species	Variable				
	Shoot Dry Weight Gain (g)	Root Dry Weight Gain (g)	Total Fresh Mass Gain (g)	Total Dry Mass Gain (g)	% Reduction in Total Dry Mass
None (Un-Inoculated)	1.91 AB	0.19 AB	24.47 B	2.34 B	26.13 A
Azospirillum brasiliense	1.90 AB	0.17 A	22.59 AB	2.10 AB	27.80 A
Bacillus amyloliquifaciens	1.84 AB	0.20 AB	22.95 AB	2.10 AB	43.13 B
Curvularia protuberata	1.66 A	0.17 A	20.00 A	1.98 AB	26.29 A
Glomus intraradices	2.13 B	0.21 B	20.24 A	1.82 A	27.46 A
Trichoderma harzianum	1.76 AB	0.16 A	20.46 AB	1.94 AB	24.84 A

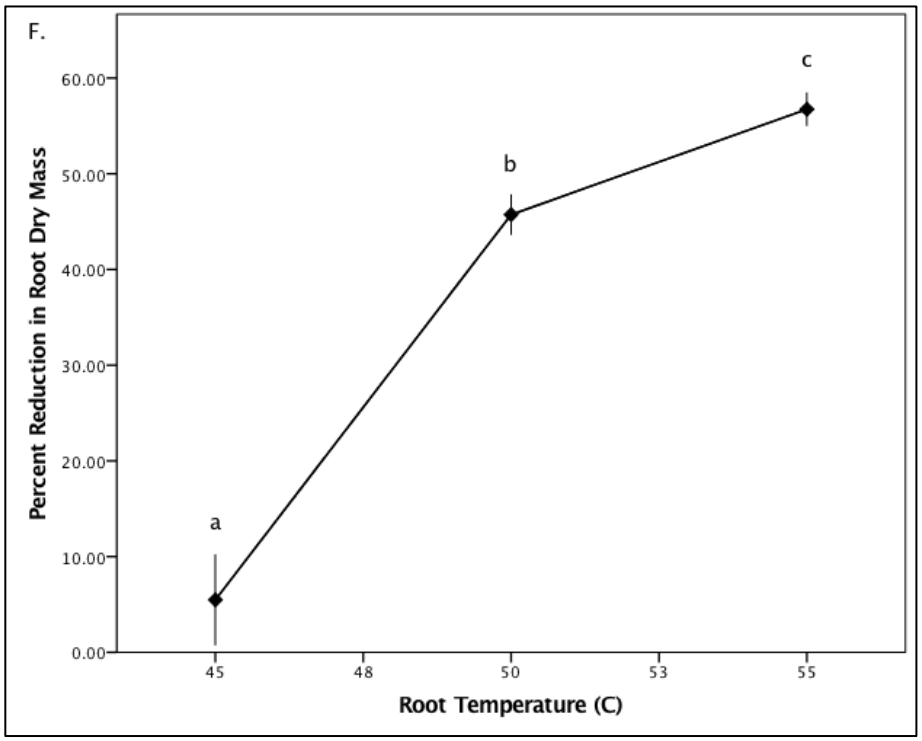

A.
b
b
a
a
2.60
2.40
2.20
2.00
1.80
1.60
1.40
1.20
Shoot Dry Mass Gain (g)
35
40
45
50
55
Root Temperature (C)

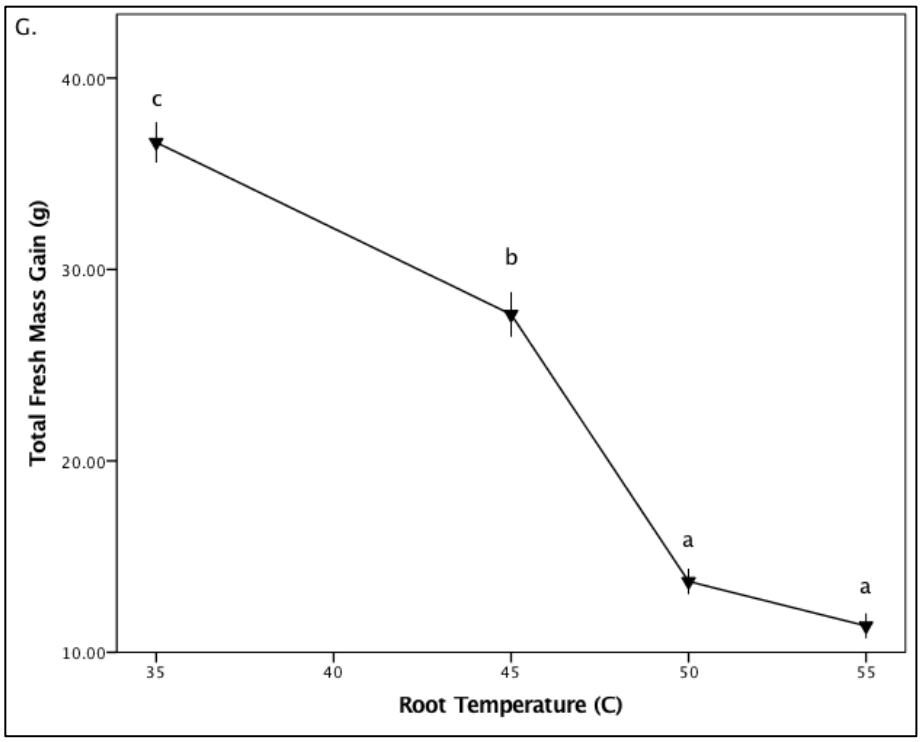

B.
b
b
a
50.00
40.00
30.00
20.00
10.00
0.00
Percent Reduction in Shoot Dry Mass
45
50
55
Root Temperature (C)

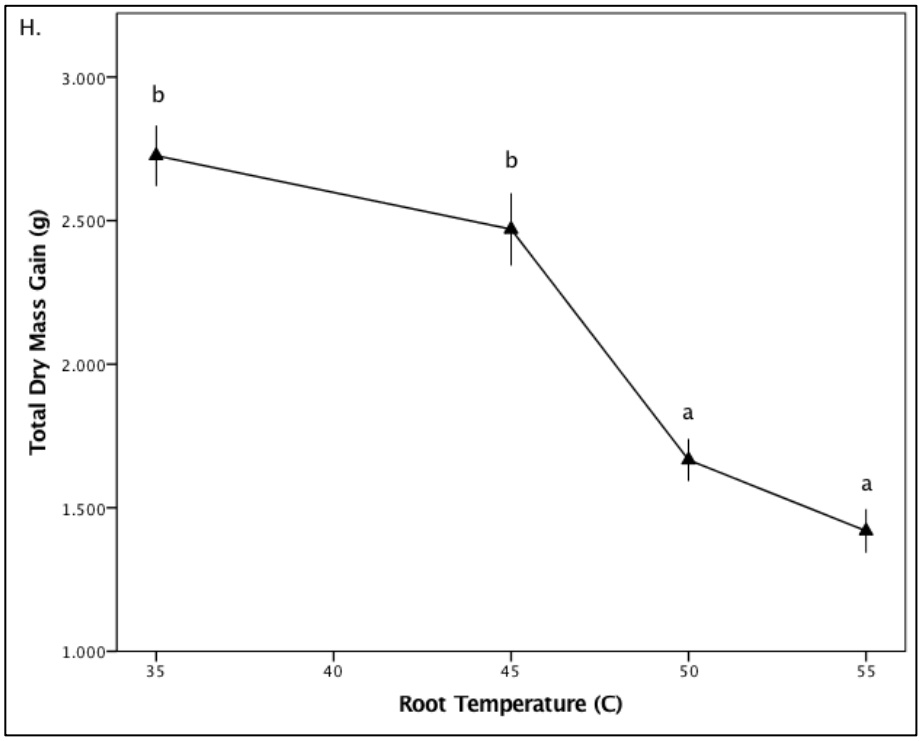

C.
4.00
3.00
2.00
1.00
0.00
Root Fresh Mass Gain (g)
c
b
a
a
35
40
45
50
55
Root Temperature (C)


D.
70.00
60.00
50.00
40.00
30.00
20.00
10.00
Percent Reduction in Root Fresh Mass
c
b
a
45
50
55
Root Temperature (C)


E.
b
b
a
a
0.30
0.25
0.20
0.15
0.10
0.05
Root Dry Mass Gain (g)
35
40
45
50
55
Root Temperature (C)


F.
c
b
a
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Percent Reduction in Root Dry Mass
45
48
50
53
55
Root Temperature (C)


G.
40.00
30.00
20.00
10.00
Total Fresh Mass Gain (g)
c
b
a
a
35
40
45
50
55
Root Temperature (C)


H.
3.000
2.500
2.000
1.500
1.000
Total Dry Mass Gain (g)
b
b
a
a
35
40
45
50
55
Root Temperature (C)

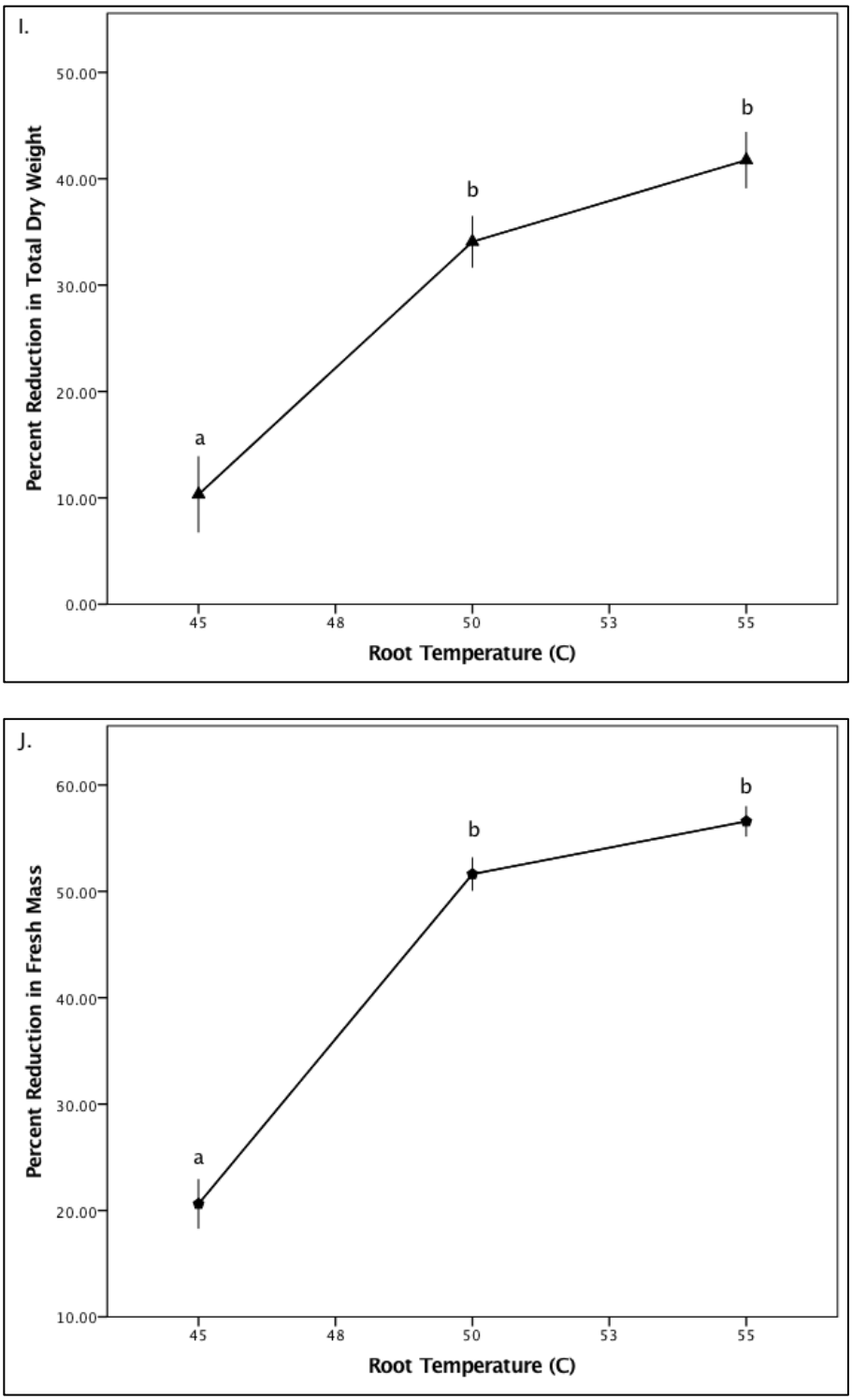


Figure 4.2. Effects of root temperature on tomato (*Solanum lycopersicum*) whole plant, shoot, and root mass gain and the percent reduction in total, shoot, and root mass gain from plants grown at root temperatures of 35°C across inoculant species. Plants were grown at elevated root temperatures for 8 h d^{-1} over a 10 d period. Letters indicate mean separation via Tukey's $_{HSD}$ ($p \leq 0.05$) and error bars represent +/-1 SE.

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